DOCTOR OF MEDICINE

Metabolic Syndrome, Weight and Cardiovascular Co-Morbidities

A Randomised Study Comparing the Effect of Three Dietary Approaches on Cardiovascular Risk in Subjects with the Metabolic Syndrome

Mukhtar, Rasha

Award date:
2014

Awarding institution:
University of Bath

Metabolic Syndrome, Weight and Cardiovascular Co-Morbidities

A Randomised Study Comparing the Effect of Three Dietary Approaches on Cardiovascular Risk in Subjects with the Metabolic Syndrome

Rasha Younis Abdalla Mukhtar

A thesis submitted for the degree of

Doctor of Medicine

University of Bath

School for Health

November 2013

Abstract

Introduction:

The metabolic syndrome is a cluster of disorders (obesity, dyslipidaemia, hyperinsulinaemia and hypertension) which individually or collectively lead to an increase in the risk of cardiovascular disease. Over the years it has been associated with endothelial dysfunction, raised markers of chronic inflammation, insulin resistance and clotting dysregulation.

Studies have shown that the prevalence of the metabolic syndrome in adults over the age of 20 years to be 24%, with approximately 12 million adults within the United Kingdom fulfilling the criteria for diagnosis. Numbers of individuals with the metabolic syndrome continue to rise following population trends of increasing sedentary lifestyle, high calorie intake, smoking, and stress. Associated is an increase in obesity, type 2 diabetes, cardiac disease, stroke and death. The increase is such that we can no longer be complacent about how we address the metabolic syndrome or its associated components.

The management of the metabolic syndrome is varied and includes alterations in diet, physical exercise, and oral medication. It is well documented that a 10% reduction in weight leads to reductions in lipid abnormalities, diabetes and diabetes-related deaths, other total morbidity and deaths. Many dietary regimens have been postulated to benefit not only weight gain but improve cardiovascular risk. To address this we investigated the effect three different diets (low fat; low carbohydrate, high fat; and low glycaemic load) had on the metabolic syndrome to assess whether it is the changes in dietary caloric or macronutrient intake, or overall weight loss that had the greater influences on those aspects of metabolic syndrome which could potentially reduce cardiovascular risk.

Our primary outcome was to identify which diet had the greatest effect on weight. The secondary outcome was to identify the changes seen in the parameters which make up the components of the metabolic syndrome. Tertiary outcomes included changes in cardiovascular risk, inflammatory markers and cytokines.

Methods:

One hundred and twenty one individuals fulfilling the criteria for metabolic syndrome were recruited to take part in a randomised controlled dietary intervention study. They were allocated to one of three weight-reducing dietary regimens and followed for twelve months: 1) a traditional low fat, carbohydrate loaded hypocaloric diet (LFD), 2) a low glycaemic load hypocaloric diet in which the fat intake was primarily in the form of monounsaturated fats (LGL), and 3) a high fat, low carbohydrate ad libitum diet (LCD).Anthropometric measurements, and blood samples for markers of insulin resistance, lipids, and adipocytokines were regularly taken.

Statistical analysis was completed using analysis of variance to compare the differences in results between the three groups at each of the study time intervals and the paired student t-test for within group comparisons. Statistical significance was considered to be $p<0.01$ for differences between the groups. Subjects included within the analysis were those who completed the twelve months of the study. An intention to treat analysis of weight loss was included to compare the overall outcome.

Results:

Per protocol analyses showed that all three groups lost significant amounts of weight at 3, 6, and 12 months (all $p<0.001$) with reductions for LGL and LCD being greater than for LFD (Table 1). Reductions in waist circumference were significant among the study groups.

At baseline all subjects fulfilled the criteria for metabolic syndrome with the majority displaying three components (73%). A fall in the number of individuals with the criteria for metabolic syndrome was noted in all three groups with only 17% in

LCD, 50% in LFD and 38% in LGL still displaying 3 or more components of the syndrome.

Table 1: Changes in anthropometric measures, lipids and blood pressure among the study group

	Low Carbohydrate	Low Fat	Low Glycaemic Load
Weight loss (kg)			
3 months	-8.4 ± 4.4♥	-4.0 ± 3.0♥	-6.3 ± 3.5♥
6 months	-9.7 ± 6.1♥	-4.6 ± 4.5♥	-7.7 ± 4.5♥
12 months	-8.9 ± 8.1♥	-4.4 ± 5.9♥	-6.7 ± 6.3♥
Waist reduction (cm) ((cm)			
3 months	-7.3 ± 13.3♥	-4.7 ± 2.1♥	-6.5 ± 3.5♥
6 months	-9.7 ± 4.9♥	-6.7 ± 3.6♥	-7.8 ± 4.5♥
12 months	-10.4 ± 7.0♥	-7.8 ± 4.6♥	-6.6 ± 6.3♥
Total-cholesterol (mmol/l)			
3 months	0.18 ± 0.73	-0.01 ± 0.59	-0.37 ± 0.63*
6 months	0.32 ± 0.84	-0.09 ± 0.65	-0.24 ± 0.78
12 months	0.19 ± 0.92	0.05 ± 0.62	-0.22 ± 0.71
HDL-cholesterol (mmol/l)			
3 months	0.23 ± 0.16♥	-0.00 ± 0.16	0.02 ± 0.18
6 months	0.26 ± 0.35*	0.06 ± 0.21	0.08 ± 0.16*
12 months	0.27 ± 0.32*	0.09 ± 0.19♦	0.18 ± 0.32*
LDL-cholesterol (mmol/l)			
3 months	0.21 ± 0.64	-0.08 ± 0.45	-0.20 ± 0.53♦
6 months	0.28 ± 0.72	-0.02 ± 0.51	-0.07 ± 0.61
12 months	0.12 ± .076	0.05 ± 0.53	-0.20 ± 0.63
Triglycerides (mmol/l)			
3 months	-0.59 ± 0.84*	-0.36 ± 0.71*	-0.34 ± 0.55♥
6 months	-0.47 ± 1.05♦	-0.41 ± 0.87♦	-0.51 ± 0.73♥
12 months	-0.41 ± 0.77♦	-0.36 ± 0.99	-0.37 ± 0.75*
HOMA-IR (%)			
3 months	-23 ± 23♥	-19 ± 30♥	-20 ± 26*
6 months	-24 ± 41♦	-23 ± 26♥	-18 ± 37*
12 months	-28 ± 26*	-16 ± 32*	-28 ± 23♥
Systolic Blood pressure			
3 months	-3.3 ± 14.4	-4.0 ± 15.0	-2.5 ± 17.2
6 months	-7.3 ± 13.3♥	-3.5 ± 15.1	-7.8 ± 16.7♦
12 months	-8.8 ± 15.8♦	-2.5 ± 12.2	-8.7 ± 16.3

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to within group changes from baseline)

Mean baseline total-cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were 5.69, 1.35, 3.41 and 2.01mmol/l respectively. Total and LDL-cholesterol (-0.37, -0.24, -0.22mmol/l; p<0.01 at 3 months) and (-0.20, -0.07, -0.20; p<0.05 at 3 months) fell with LGL at 3, 6 and 12 months, rose with LCD but was not

significant (0.09, 0.22, 0.12; all p=NS) and (0.57, 0.25, 0.19; all p=NS) for the same time intervals. HDL-cholesterol improved in all three groups with rises significant in the LCD and LGL groups. A significant fall in triglycerides was noted in all three diets with the changes persisting up to 12 months for LCD and LGL.

Systolic blood pressure fell numerically in all three groups although changes were only significant among the LCD group (-3.3, -7.3, & -8.8mmHg, $p<0.001$ at 6 months).

Reductions in insulin resistance was seen in all three groups with a fall in HOMA-IR of 19, 23, & 16% (all $p<0.01$) for LFD, 20, 18 & 28% (all $p<0.01$) for LGL, and 23, 24, & 28% (all $p<0.01$) for LCD at 3, 6 and 12 months respectively.

At 3, 6 and 12 months adiponectin rose 9.5, 9.2 & 28.8% (p=NS) for LFD, 9.5, 31.2 & 29.6% ($p<0.001$ at 6 and 12 month) for LGL, and 24.9, 49.6 & 41.2% (all $p<0.01$) for LCD. At 3, 6 and 12 months leptin levels fell by 15, 17 & 9% (all $p<0.05$) for LFD, by 33, 32 & 29% (all $p<0.001$) for LGL, and 35, 39 & 24% (all $p<0.01$) for LCD. There were no correlations between leptin and adiponectin. Changes among the other cytokines and inflammatory markers were non-specific and failed to show any correlation to anthropometric measures or the other cytokines.

Conclusion:

All three dietary interventions produced clinically meaningful reductions weight and in other anthropometric measures. There was a fall in the number of individuals who met the criteria for metabolic syndrome in all three groups with reductions being highest among the LCD group. Markers of insulin resistance improved among the three study groups. The LCD was superior at blood pressure reduction, cardiovascular risk reduction and positively influencing cytokines and inflammatory markers. The LGL group demonstrated more favourable effects on lipid profile in addition to its positive role on blood pressure, cardiovascular risk reduction and some of the inflammatory markers. The long term effect of such interventions on cardiovascular outcomes needs further study.

Acknowledgements:

I owe much gratitude to a number of individuals who have expressed unrelenting faith, encouragement and support in this endeavour. I owe much gratitude to Professor John Reckless for his academic direction, opinions and providing me with the opportunity and funding to undertake this research.

I also owe gratitude to Dr Julia Reid for her patience and support during the long years of data collection, blood processing and analysis. I also owe her for spending long hours running all the ELISAs used in this study.

I would like to express gratitude to those who willingly gave of their time to participate and made this study possible.

I would like to extend my thanks to Drs Tony Robinson and Lynn Higgs for their support, and all the staff at the Wolfson Centre, Royal United Hospital who looked after the participants on their visits and provided me with a non-ending supply of caffeine. I would also like to acknowledge Professor Rudy Bilous and my colleagues at James Cook University Hospital, who gave me the time, space and support so that this project may be completed

I also need to thank my family and friends, most notably but not in any way limited to, my mother, father, brothers, my cousin Mawahib, and Fatyha, who would regularly call to support, harass, and provide many words of advice and wisdom when I was about to give up.

Words cannot express appreciably enough what their love, support, encouragement and faith has meant to me.

Conference Abstracts:

1. The Effect of Three Dietary Regimens on Insulin Resistance in Metabolic Syndrome Subjects
   RYA Mukhtar, J Reid, HF Fishlock, GJ Taylor, JPD Reckless
   1737-P, American Diabetes Association

2. Low Glycaemic Load, High-Protein Diets Produce More Favourable Changes in Leptin Levels Than Traditional Diets among Subjects with Metabolic Syndrome
   RYA Mukhtar, J Reid, HF Fishlock, GJ Taylor, JPD Reckless
   P2-411 Obesity: Clinical Assessment & Treatment, ENDO 2009

3. The Effect of Three Dietary Interventions on PAI-1 among Metabolic Syndrome Subjects
   J Reid, R Mukhtar, H Fishlock, G Taylor, J Reckless
   Atherosclerosis Supplements 2009, Vol. 10, Issue 2
   International Symposium for Atherosclerosis

4. Low glycaemic load, high-protein diets produce more weight loss than traditional diets in subjects with metabolic syndrome
   International Congress for Obesity
   Obesity Reviews 7 (Suppl. 2) (2006) 118–352

5. Low glycaemic load, high-protein diets have a more beneficial impact on lipid profiles than traditional diets in subjects with metabolic syndrome
   International Congress for Obesity
   Obesity Reviews 7 (Suppl. 2) (2006) 118–352

Table of Contents

List of Tables

List of Figures

ABBREVIATIONS

ADA	American Diabetes Association
ALT	Alanine transaminase
ATP III	Adult Treatment Panel III
BAT	Brown adipose tissue
BMI	Body mass index
CHD	Coronary heart disease
CRP	**C**-reactive protein
CTT	Cholesterol Treatment Trialists'
CVD	Cerebrovascular disease
EASD	European Association for the Study of Diabetes
EDTA	2-ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FFA	Free fatty acids
FPG	Fasting plasma glucose
GI	Glycaemic index
GL	Glycaemic load
GLUT	Glucose-transporting protein
GP	General Practitioner
HDL	High density lipoprotein
HOMA	Homeostasis model assessment
hsCRP	High sensitivity C-reactive protein
IDL	Intermediate density lipoprotein
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IHD	Ischaemic heart disease
LCD	Low carbohydrate diet
LDL	Low density lipoprotein
LFD	Low fat diet
LGL	Low glycaemic load

LPL	Lipoprotein lipase
MI	Myocardial infarction
MS	Metabolic syndrome
MHRA	Medicines and Healthcare products Regulatory Agency
MUFA	Monounsaturated fatty acids
NCEP	National Cholesterol Education Programme
NEFA	Non-esterified fatty acids
NHANES	National Health and Nutrition Examination Surveys
NS	Not significant
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activating inhibitor type 1
PVD	Peripheral vascular disease
RBP-4	Retinol binding protein -4
RUH	Royal United Hospital
SCOUT	Sibutramine in Cardiovascular Morbidity & Mortality Outcomes Trial
TG	Triglyceride
UK	United Kingdom
US	United States
VLCD	Very low calorie diet
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WHO	World Health Organisation
WHR	Waist-hip Ratio

1. CHAPTER 1: Introduction to Obesity and Cardiovascular Risk

1.1. Obesity

Obesity is defined as an abnormal or excessive accumulation of fat to a level that may negatively impact on health. It is the physiological response to an environment in which energy intake exceeds energy output.

The prevalence of obesity worldwide has progressively risen over the past century reaching epidemic proportions. Data published by the World Health Organization (WHO) in 2011 showed that the estimated prevalence of obesity had more than doubled since 1980 with 1.5 billion adults classified as overweight, of whom approximately 500 million fell within the obese category. Without intervention these figures are expected to rise to 2.3 billion individuals classified as overweight and 700 million as obese by 2015[582].

Initially an affliction of developed countries, obesity is increasing in developing countries particularly within urban areas where cultural and behavioural changes have altered as they adopt the life styles of developed countries.

1.1.1. Historical

Many beliefs and theories regarding the evolution of obesity exist. One argument claims obesity to be the disease of modernisation, claiming that it barely existed in the ancient world where man depended on scavenging skills to overcome food scarcity, disease, and a hostile environment[78]. Others believe that the accumulation of fat was due to environmental and genetic adaptations equipping individuals with the best means of survival for their circumstances.

For thousands of years, obesity was exceptional, and poorly studied although figurines of abdominally obese females such as the 'Venus of Willendorf' (Figure 1)

have been present from as far back as 30,000 years. The history of these statuettes is hotly debated. Some believe them to represent the earth goddess, others classified them as symbols of fertility, while a third group have implied that these palm sized figurines were the predecessors of *Playboy* magazine [225].

Figure 1: Venus of Willendorf

Accessed online [5]

Man originally developed as a hunter gather. No anthropometric studies of these groups have shown evidence of obesity in these individuals. They are considered to have led active lives and enjoyed a highly varied diet rich in protein but limited in carbohydrate, based on wild game and plants which they would procure. Eating patterns were cyclical depending on luck and availability. With the development of settlements and domestication, a steady nutritional supply became available based on crops which were higher in carbohydrate content, and lower in protein and fats. This new calorie-richer, but less varied diet led to a higher morbidity and reduced lifespan. As new communities flourished and sub-divided into social stratifications, an elite upper class evolved which maintained a constant nutritional supply with minimal physical exertion breaking the feed-fast cycle and linked affluence to obesity [319].

One of the first documentations of the vices of obesity and diet comes from the ancient Greeks. Hippocrates described many ailments which he ascribed to poor health and made an observation which later became the basis for energy intake balance:

'It is very injurious to health to take in more food than the constitution will bear, when, at the same time one uses no exercise to carry off this excess . . . .

For as aliment fills, and exercise empties the body, the result of an exact equipoise between them must be to leave the body in the same state they found it, that is, in perfect health'.

His son-in-law, Polybus went on to suggest that diet and exercise would be an effective measure for overweight individuals[225]. Acceptance of obesity as a medical phenomenon requiring treatment has been slow. Tobias Venner was the first physician to use the word 'obesity' in a medical context. He discussed the need for treatment in his publication in 1660 where he recommended the waters at Bath as treatment, stating:

'beneficial to such individuals: to make slender such bodies as are too grosse . . . Wherefore let those that feared obesity, that is, would not wax grosse, be careful to come often to our Baths: for by the use of them, according as the learned Physician shall direct, they may not only preserve their health, but also keep their bodies from being unseemly corpulent'[556].

With the commencement of the industrial revolution, an increase in body mass, particularly muscle, was linked to improvements in health, greater productivity and efficiency. Developed countries were then still struggling with poverty and malnutrition and to help overcome this poor children's' diets were supplemented with sugar and fat following observations by Lord Boyd Orr [400;401]. Consequently, the population progressively increased in height and weight and then began expanding outwards, once the full potential to grow in height was realised, and individuals began accumulating weight and girth[220].

Initially body weight was compared to an "ideal body weight" derived from tables maintained by insurance companies as their measure for obesity. In the 1980s, the Body Mass Index (BMI), a statistical measure which compares a person's weight and height, replaced this. Parameters were fixed with "normal" BMI defined as 20-<25kg/m^2 and "obesity "defined as BMI $\geq$ 30 kg/m^2 for both men and women. Although a useful tool for the measurement of adiposity within a population base,

there are limitations to the BMI as it is dependent on weight, not adiposity, and thus does not correspond to the same level of fatness in different individuals (e.g. athletes have raised BMI due to muscle mass), and the cut off parameters for morbidity and mortality risk vary between ethnic groups, (e.g. a cut off of 23 rather than <25 for Asians who are more prone to visceral obesity and higher cardiovascular risk).

The United States is one of the countries with the highest degree of obesity. To help address this, dietary and life-style modifications in the form of advice on caloric restrictions, reduction in fat intake and exercise were introduced. Despite reductions in fat content, an increase in low calorie food, and weight loss clubs, the average population weight has continued to rise. The easy availability of transport and gadgets which reduce work load encourage a more sedentary lifestyle with less requirement for energy expenditure. In combination with the easy availability and low cost of energy dense food, these provide an environment conducive to weight gain and obesity.

By the year 2000, the human race "achieved" an historical landmark, where for the first time in human evolution the number of overweight (BMI >25kg/m^2) adults surpassed the number of those who were underweight (BMI <18.5kg/m^2). Accompanying the increase in weight are a number of health issues including cardiovascular disease, arthritis, cancer and respiratory dysfunction which result in a greater burden both physical and economical upon society. Cardiovascular disease and its associated risk factors, has been extensively studied and over the years has been interlinked in what is now known as the metabolic syndrome.

1.1.2. Rising Prevalence of Obesity

Since the 1960s, the prevalence rate of overweight individuals has increased by 50% across all population groups. Now, one in two adults and one in four children are overweight[60]. The National Health and Nutrition Examination Survey (NHANES) is a national program developed in the early 1960s in the United States looking at a number of health issues among the population. It consists of sequential surveys which focus on the nutritional and health aspects of the population which is meant to collect data that will help look into and address issues related to the health of the nation. Data from 2003-2004 NHANES estimate that two thirds of the adult US population are overweight, (BMI $\geq$25 kg/m$^{2)}$; with just over 32% meeting the criteria for obesity (BMI $\geq$30 kg/m^2), while 4.8% fit in the extreme obesity category (BMI $\geq$40 kg/m^2) [397].

Data from the NHS information centre state that in the UK approximately 35% of men and women aged 16 years and over were considered to be overweight while a further 26% were obese (BMI $\geq$30 kg/m^2)[4]. Furthermore, the rise in obesity prevalence has not been the privilege of adults only. Childhood and adolescence obesity is on the rise, promoting the onset of risk factors for chronic disease in their youth. Up until the 1970s, obesity prevalence in children aged 2 to 19 years was static at approximately 5%[268]. Since 1980, it has increased threefold in individuals aged 20 years or younger. Current estimates report the prevalence of obesity to be 17.1% among the teenagers of the United States with figures for the United Kingdom being little different.

Despite obesity becoming a universal epidemic, certain patterns and discrepancies were observed which affected particular demographics more than others. In developed countries obesity particularly in females was associated with lower income and education, whereas the reverse was true for developing countries. NHANES data identified that black and/or Hispanic females were generally larger than their Caucasians counter-parts even when income and education were accounted for. UK data similarly identified individuals from Caribbean, Pakistani or Irish descent to be more likely be obese with the prevalence higher among females[445](Table 2). Unlike females, males did not seem to be greatly affected by any particular parameter apart from a tendency to an increased girth with rising wealth.

Epidemiological studies in children were similarly non-polarised although the general acceptance appears to be that in developed countries children from lower socioeconomic backgrounds tended to have higher BMIs than their privileged, better educated, and financially comfortable colleagues[55;360;445] .

Table 2: Prevalence of obesity in adults

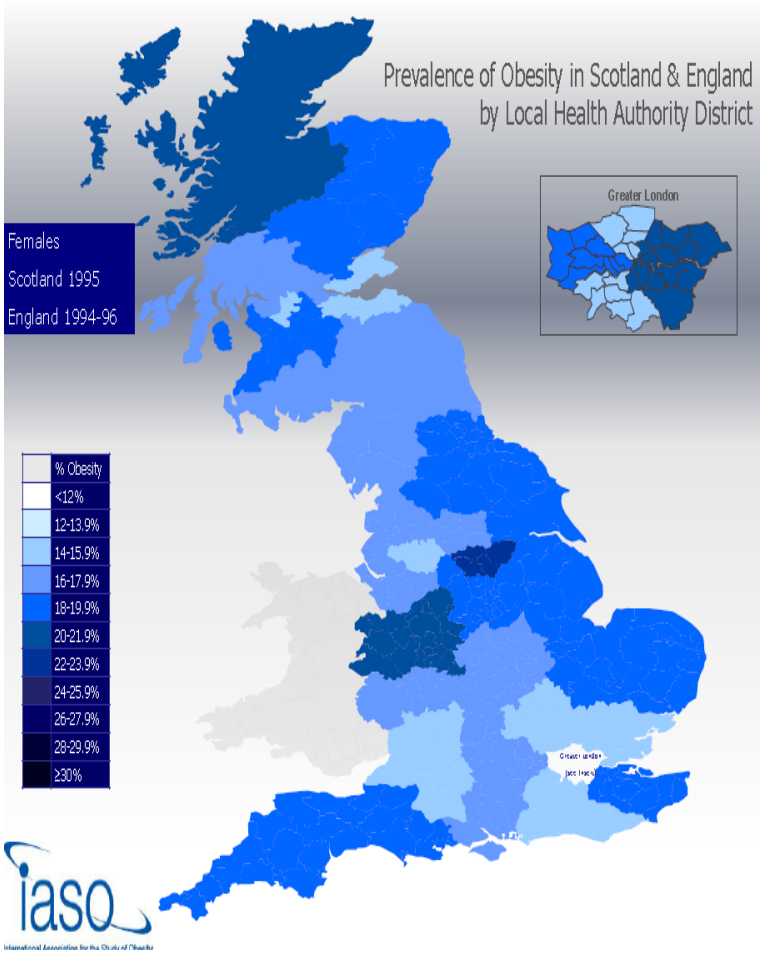
	Men (n = 3204)	Women (n = 3699)
Black Caribbean	18.3	31.9
Indian	11.9	19.6
Pakistani	12.6	25.6
Bangladeshi	5.4	9.5
Chinese	6.2	4.5
Irish	20.4	21.2
General population	18.9	20.9

Prevalence of obesity (% with BMI > 30 kg m^{-2}) in adults (16 years and over) by ethnic group in Health Survey for England 1999. Adapted from[445]

Certainly within developed countries geographical variations in the distribution of obesity were noted. In the US the prevalence is highest within the central southern states, areas renowned for higher numbers of both blacks and Hispanics, as well as a greater percentage of individuals classified as being low income earners.

Within the UK, the highest prevalence of obesity is in Scotland followed by Northern Ireland, England and finally Wales (Figure 3 & Figure 4). Similarly to the US, areas with well documented social deprivation including the north east, west midlands and east London had a higher prevalence of obesity[24]. In contrast, developing countries have seen the distribution of obesity rising within their wealthier, urban regions[266;492]. What is irrefutable is that obesity is increasing worldwide and if unregulated will be a large burden both financially and physically upon society (Figure 3 - 6).

Figure 2: UK Obesity Trends 1995 - 2003 (Females)



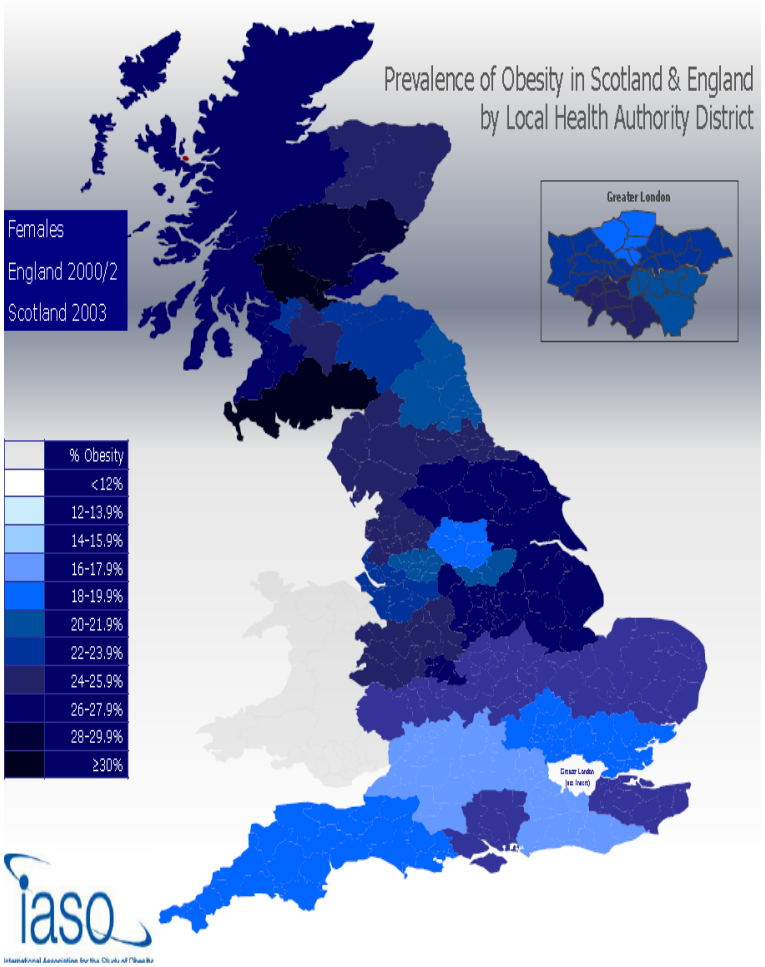
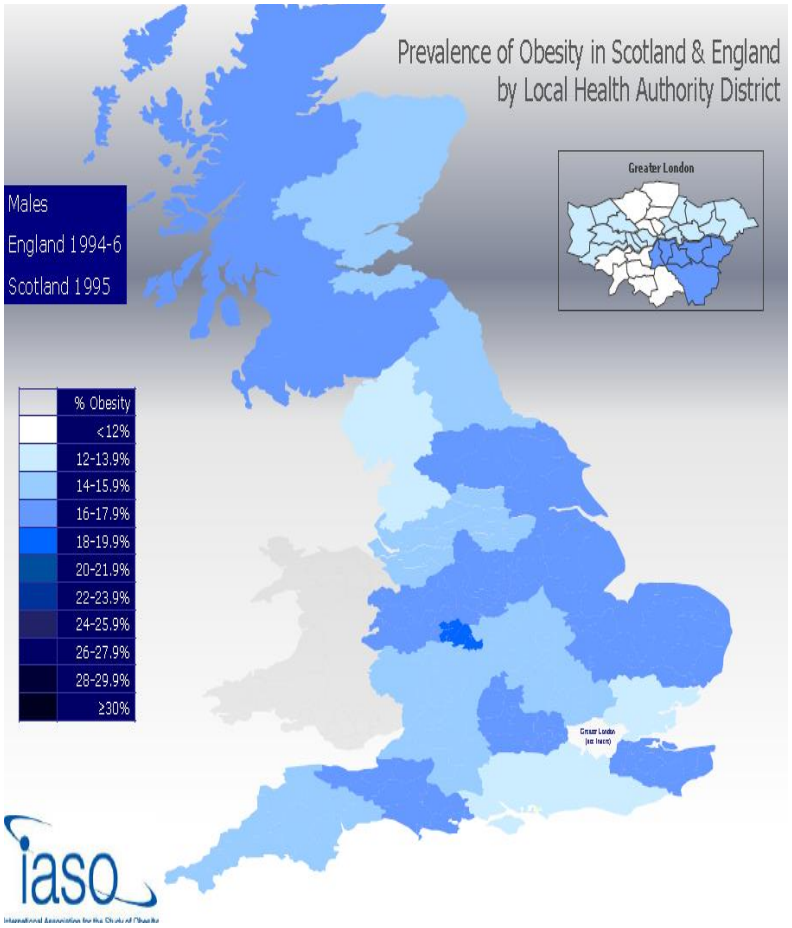
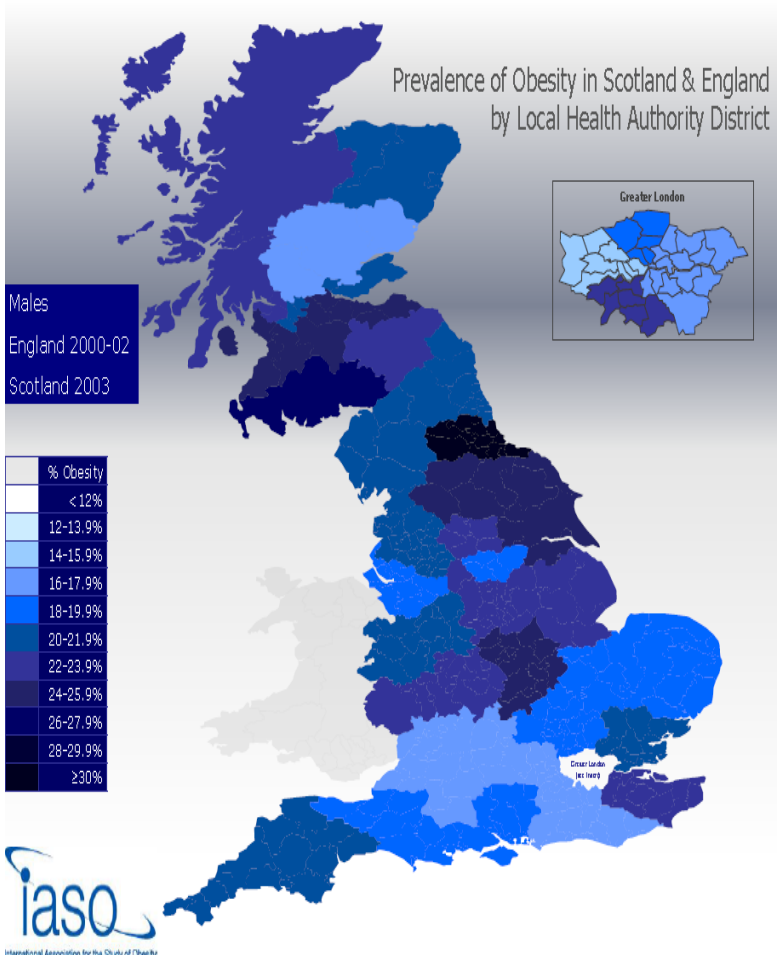


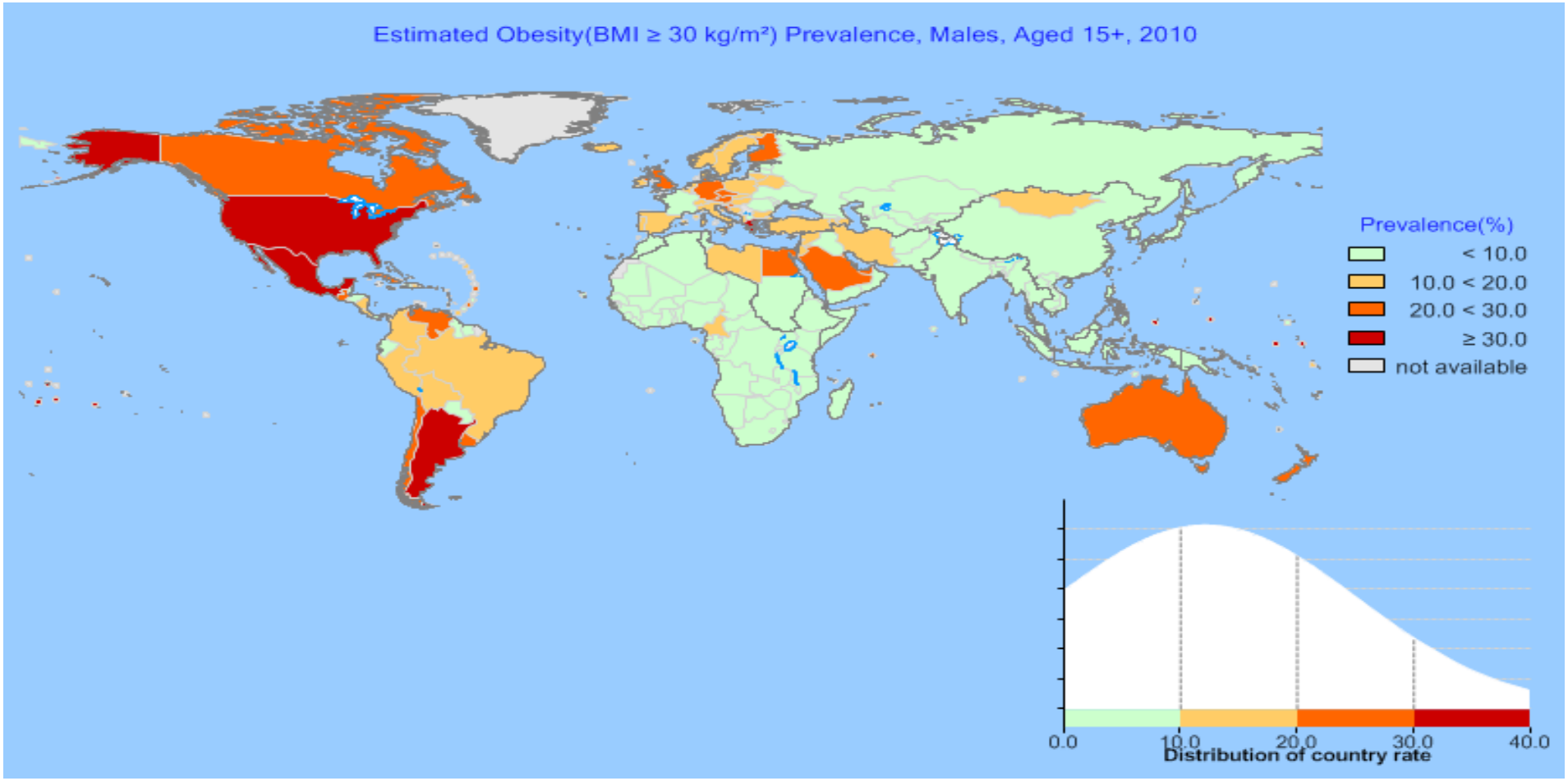
Figure 3: UK Obesity Trends 1995 - 2003 (Males)





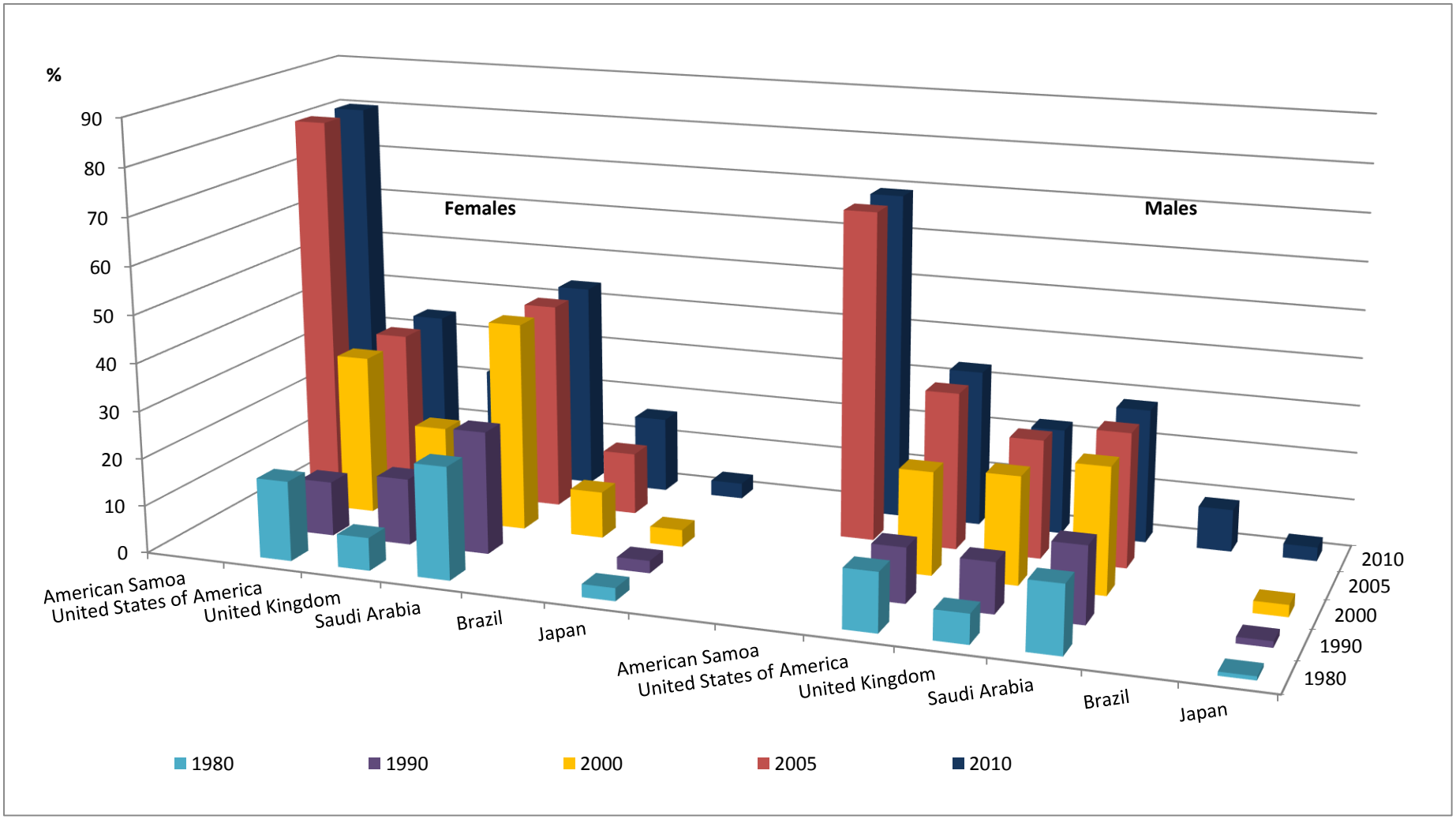
Taken from: http://www.iaso.org/site_media/library/resource_images/Trend_in_UK_by_Health_Authority_April_2012.pdf

Figure 4: Prevalence of obesity (BMI>30kg/m2) among males 2010



These are data from the WHO website showing that most developed countries have a >20% prevalence of obesity (orange and red areas) whereas in Asian and African countries, and the Far East levels are less than 10%[583]

Figure 5: Rising prevalence of obesity worldwide from 1980 - 2010



Changes in obesity prevalence in a 30 year span the West, East and Middle East (Adapted from data on the WHO website where available

1.2. Effects of Obesity on Individuals and Society

Obesity is now widely recognized as one of today's leading health threats in most countries and as a major risk factor for type 2 diabetes, cardiovascular disease, and hypertension. Mortality data from NHANES report that the number of deaths in the USA in 2000 attributed to obesity was 112,000 in excess of that in individuals with a normal BMI[158]. The effect of obesity on an individual can be profound affecting not only general health but social and psychological well-being. Listed below are a series of such factors:

1. An increase in cardiovascular disease including myocardial infarction, and stroke.
2. An increased incidence of Type 2 Diabetes
3. An association with neoplastic disorders (i.e. colon, breast and endometrial).
4. Gastrointestinal dysfunctions (i.e. gallstones, gastro-oesophageal reflux, and non-alcoholic fatty liver disease)
5. Physical dysfunction due to arthritis, associated pain and reduced mobility
6. Obstructive sleep apnoea
7. Social withdrawal, isolation, and depression
8. Economic pressures secondary to loss of earnings due to decreased ability to work, food costs and clothing

In the Prospective Studies Collaboration, a meta-analysis of 57 studies, the lowest incidence for all-cause mortality was at a BMI of 22.5 – 25kg/m^2 with each categorical increment in BMI associated with an increase in CV mortality by 40%, neoplastic risk by 10%, and overall mortality by 30%[431].

Type 2 diabetes and obesity have been closely linked, with the latter repeatedly blamed for the rising incidence of the former. Individuals with a BMI of 25kg/m^2 have a 5 times greater risk for developing diabetes than those with a BMI <20kg/m^2 with increments rising up to 93 times for those with a BMI >35kg/m^2. The two conditions share causative factors, but do not necessarily lead to one another. However the development of diabetes/insulin resistance in the obese leads to an exponential rise in CV mortality[39].

1.2.1. Financial Burden of Obesity

Prevalence of overweight and obesity has escalated during the past few decades, and this is contributing to the overall cost of health care. These costs do not only include expenses related to illness and medical treatment but also further out-reach costs including reduced productivity due to higher levels of absence from work, increased social care costs and higher government expenditure on disability and unemployment benefits. In 2002, the House of Commons Health Select Committee calculated the annual bill for the management of the obese and their co-morbidities to be £3.3–3.7 billion with figures doubling to £6.6–7.4 billion if the overweight (BMI 25–30kg/m^2) were also taken into account. If broken down into segments, a sixth (£991–1124 million) is incurred in the direct management of obesity, whilst the remainder (£5.5–6.2 billion) is spent on the secondary consequences.

A further financial burden is as a consequence of loss of earnings, and income support. Lost earnings attributable to obesity were estimated to be £2.3–2.6 billion of which £1.3–1.5 billion is certified sick leave. If obesity levels continue to rise then the predicted National Health Service spending will almost double by 2050. In the USA, a 1998 article demonstrated that 17% of the costs of CHD were related to obesity with the overall medical expenditure estimated at $78.5 billion, half of which was covered by health insurance companies including Medicare and Medicaid. Data from 2009 estimated the annual medical cost of obesity to be approximately $147 billion per year (10% of the total US Healthcare bill). These figures are predicted to rise by a further $48-66 billion per annum by 2050 if obesity continues to rise[157].

1.3. Metabolic Syndrome

Obesity is well recognised as the presence of excessive body fat. Depending on where the excess fat has been deposited divides obesity into subcutaneous (peripheral) or visceral (central) adiposity. It is the visceral fat which is believed to be most influential in cardiovascular risk. Visceral fat is generally deposited within the abdominal cavity surrounding the organs within. The metabolic dysfunction created by excess visceral fat mass influences multiple factors of disease risk. These have been identified from as far back as the 1920's and eventually grouped together into a cluster including abdominal obesity, dyslipidaemia, hyperinsulinaemia and hypertension, all of which may independently or collectively lead to an increase in the risk of cardiovascular disease. In 1988, Gerald Reaven presented the accumulation of his work at the "Banting" lecture identifying this group of risk factors as "Syndrome X" and proposing that insulin resistance was the underlying causative element. At this point he did not include obesity in his definition[441].

Over the years the clustering has been referred to as Reaven's syndrome, the deadly quartet, insulin resistance syndrome, and more recently, the metabolic syndrome (MS). The presence of all of the components of MS, in an individual increases the risk of cardiovascular morbidity and mortality by two-threefold. Over the years MS has also been associated with hypertriglyceridaemia, low HDL cholesterol, hyperuricaemia, abnormalities of fibrinolysis and clotting, endothelial dysfunction, raised markers of chronic inflammation, polycystic ovary syndrome and obstructive sleep apnoea[103;560;570].

With the global rise in overweight and obese individuals, following population trends of increasing sedentary lifestyle, a disproportionately high calorie intake, smoking, and stress, the prevalence of MS has risen dramatically and associated is the threat of an increase in atherosclerotic disease, type 2 diabetes and death[39]. The increase is such that we can no longer be complacent about how we address the metabolic syndrome or associated components.

1.3.1. Metabolic Syndrome – Diagnosis

Although accepted as a clinical entity it has been difficult to define MS as its components are abnormalities which may occur individually or collectively in varying degrees of severity. It therefore became necessary to attempt to provide a universal definition of MS in order to identify and target those individuals at risk.

The first definition was produced by the WHO in 1998. This group placed a large emphasis on insulin resistance being a major risk factor and considered it to be an essential requirement for the diagnosis in addition to two other criteria which included waist to hip ratio, hypertriglyceridaemia, low HDL-cholesterol, hypertension and microalbuminuria. The WHO criteria was criticised as it was felt to be impractical in clinical or epidemiological settings to measure insulin resistance through clamp methods. Further dissention existed on what was the appropriate measure for obesity (i.e. waist to hip ratio versus abdominal girth), and the inclusion of microalbuminuria as one of the criteria[14]. In 1999, the European Group for Study of Insulin Resistance (EGIR) produced a modification of the WHO definition excluding those with type 2 diabetes as insulin resistance was considered to be a risk factor for diabetes.

Two years later, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) (Table 3) introduced an alternative set of criteria for defining the metabolic syndrome. They aimed to identify individuals at risk of developing cardiovascular disease who thus qualified for intervention of one form or another. Insulin resistance, although a component, was no longer an essential criterion for diagnosis and was identified as a fasting glucose sample rather than the more laborious clamps, or glucose tolerance test which are not standardized measures of testing in general clinical practice. The ATP III required the presence 3 of 5 factors as the basis for diagnosis, assuming all risk factors were equal. These were abdominal obesity, hypertriglyceridaemia, reduced HDL-cholesterol, hypertension, and impaired fasting glucose[26].

In 2003, the American Association of Clinical Endocrinologists (AACE) modified ATP III criteria refocusing on insulin resistance. Their criteria included

impaired glucose tolerance (IGT)[1], hypertriglyceridaemia, reduced HDL-cholesterol, hypertension, and obesity. The diagnosis was left to the physician's clinical judgement with encouragement to take external factors into account including family history of CVD, type 2 diabetes mellitus, polycystic ovary syndrome, and hyperuricaemia.

Table 3: NCEP-ATP III Criteria for the diagnosis of the Metabolic Syndrome

Measure	Categorical cut-off points
Elevated waist circumference	>102cm (40in) Male >88cm (35in) Female
Elevated triglycerides	>150mg/dl (1.7mmol/l)
Reduced HDL cholesterol	<40mg/dl (1.03mmol/l) male <50mg/dl (1.3mmol/l) female
Elevated blood pressure	Systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg
Elevated fasting glucose	≥110 mg/dl (6.1mmol/l) Or on treatment for diabetes

Need 3 out of 5 to fulfil criteria to diagnose the syndrome

Although the above definitions were intended to detect the majority of individuals with MS, it soon became apparent that different sectors were being targeted by each. When the subjects in the AusDiab study were classified to have metabolic syndrome or not, three of the classifications were adopted, the WHO, EGIR, and ATP III. Each definition identified approximately 15–20% of the population to fulfil criteria for MS, but only 9.2% met the collective criteria from all three classifications[130].

In 2005, the International Diabetes Foundation (IDF) published new criteria modifying the ATP III definition aiming to establish a universal definition and method for clinical diagnosis[251].

They considered that abdominal obesity was highly correlated with insulin resistance and made the presence of abdominal obesity necessary for the diagnosis in addition to one of the four previously used criteria. IDF recognized the ethnic differences in the correlation between abdominal obesity and other MS risk factors and specified that waist measurement be specific to each racial group. With these

[1] IGT is defined a 2 hour post glucose load of >7.8mmol/l and <11.1mmol/l (in the presence of a normal or elevated fasting glucose), and impaired fasting glucose (IFG) is defined as glucose levels >6.1mmol/l and <6.9mmol/l.

recommendations abdominal obesity thresholds were set at waist circumferences ≥94 cm in men and ≥80 cm in women. For Asian populations, except Japan, thresholds were ≥90 cm in men and ≥80 cm in women; for Japanese they were ≥85 cm for men and ≥90 cm for women[13;251]. At about the same time the ADA and EASD issued their combined statement in *Diabetes Care* September 2005 reporting:-

"Our analysis indicates that too much critically important information is missing to warrant its designation as a syndrome. Until much needed research is completed, clinicians should evaluate and treat all cardiovascular risk factors without regard to whether somebody has the metabolic syndrome or not[272].

Therefore disagreements persisted as to how best to define and diagnose MS until 2009, when the major institutions once more assembled to release an updated statement and definition which no longer placed abdominal adiposity as a requisite for the diagnosis, but once more reverted to a diagnosis being established on the presence of 3 of the 5 criteria[15] (Table 4).

Table 4: International Diabetes Federation 2009 revised criteria for the diagnosis of Metabolic Syndrome

Measure	Categorical cut points
Elevated waist circumference	Population- and country-specific definitions
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator)	≥150 mg/dL (1.7mol/l)
Reduced HDL cholesterol (drug treatment for reduced HDL cholesterol is an alternate indicator)	<40 mg/dL (1.0mmol/l) for males <50 mg/dL (1.3mmol/l) for females
Elevated blood pressure (drug treatment for elevated blood pressure is an alternate indicator)	Systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg
Elevated fasting glucose (drug treatment for elevated glucose is an alternate indicator)	≥100 mg/dL (5.6mmol/l)

1.3.2. Waist-Hip Ratio

Despite the changes in diagnostic criteria for MS, abdominal girth remains a major indicator of adiposity and CV risk. Yusuf et al, in the INTERHEART study identified the waist-hip ratio (WHR) to be a strong risk factor for CHD increasing the risk by up to 2–3 times[597]. Theoretically the WHR is the ratio of waist circumference measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, to the hip circumference measured around the widest portion of the buttocks. Practically it is taken as the narrowest waist measurement to the widest hip measurement. Several sources have identified it to be a more reliable identifier for CV risk as it helps stratify individuals into "apple" (abdominal adiposity) or "pear" (fat on hips) which BMI and waist measurements are unable to discriminate. However in the WHO consensus it was felt that waist measurement was practically easier to perform and would therefore be subject to less errors[102](Table 5). Desirable WHR figures should be <0.75 for females and <0.90 for males to maintain optimum health with a 0.01 U increase in WHR, being considered to directly increase the risk of CVD by 5% (Table 6)[115].

Table 5: Recommended Waist Circumference Thresholds by Organization

Population	Organization	Recommended Waist Circumference Threshold for Abdominal Obesity	
		Men	Women
European	IDF	≥94 cm	≥80 cm
Caucasian	WHO	≥94 cm (increased risk)	≥80 cm (increased risk)
		≥102 cm (still higher risk)	≥88 cm (still higher risk)
United States	AHA/NHLBI (ATP III)*	≥102 cm	≥88 cm
Canada	Health Canada	≥102 cm	≥88 cm
European	European Cardiovascular Societies	≥102 cm	≥88 cm
Asian (including Japanese)	IDF	≥90 cm	≥80 cm
Asian	WHO	≥90 cm	≥80 cm
Japanese	Japanese Obesity Society	≥85 cm	≥90 cm
China	Cooperative Task Force	≥85 cm	≥80 cm
Middle East, Mediterranean	IDF	≥94 cm	≥80 cm
Sub-Saharan African	IDF	≥94 cm	≥80 cm
Ethnic Central and South American	IDF	≥90 cm	≥80 cm

*Recent AHA/NHLBI guidelines for metabolic syndrome recognize an increased risk for CVD and diabetes at waist-circumference thresholds of ≥94 cm in men and ≥80 cm in women and identify these as optional cut points for individuals or populations with increased insulin resistance. Adapted from[15]

Table 6: WHO, EGIR, NCEP, AHA/NHLBI and IDF definitions of Metabolic Syndrome

	WHO (1998)	EGIR (1999)	NCEP-ATP III (2001)	AHA/NHLBI (2005)	IDF (2005)
	Insulin resistance, glucose intolerance , type 2 diabetes + any 2 from below	Insulin resistance (defined as hyperinsulinaemia—top 25% of fasting insulin values among the non-diabetic population) + any 2 from below	3 of 5 criteria	Insulin resistance diagnosed on clinical judgement*	Waist measurement + 2 criteria
Waist circumference	Waist to hip ratio >0.9 Male Waist to hip ratio >0.84 Female And/or BMI >30kg/m^2	≥ 94 cm (37in) Male ≥ 80 cm (31.5in) Female	>102cm (40in) Male >88cm (35in) Female		Increased waist circumference depending on ethnic differences
Triglyceride	>150mg/dl (1.7mmol/l)	>178 mg/dl (2mmol/l) Or on treatment	>150mg/dl (1.7mmol/l)	>150mg/dl (1.7mmol/l)	>150mg/dl (1.7mmol/l) Or on treatment
HDL-cholesterol	<35mg/dL (0.9mmol/l) male <39mg/dL (1mmol/l) females	<39mg/dL (1mmol/l) Or on treatment	<40mg/dl (1.03mmol/l) male <50mg/dl (1.3mmol/l) female	<40mg/dl (1.03mmol/l) male <50mg/dl (1.3mmol/l) female	<40mg/dl (1.03mmol/l) male <50mg/dl (1.3mmol/l) female Or on treatment
Glucose	As mentioned above	≥ 6.1 mmol/l (110 mg/dl) but non-diabetic	>110mg/dl (6.1mmol/l) or diabetic	Fasting 6.1–6.9 mmol/l (110–125 mg/dl) 2hr post glucose load 8–11.1 mmol/l (140–200 mg/dl)	>110mg/dl (6.1mmol/l) or diabetic
Blood pressure	≥ 140/90mmHg	≥ 140/90 mmHg or on treatment	>130/85mmHg	>130/85mmHg	>130/85mmHg Or on treatment
Other	Microalbuminuria				

*Diagnosis of the insulin resistance is based on clinical judgement. Other factors to be considered in the diagnosis are obesity, family history of diabetes, polycystic ovary syndrome, lifestyle, advancing age and ethnic groups susceptible to Type 2 diabetes. Adapted from[14;201]

1.3.3. Metabolic Syndrome – The Expanding Problem

It is reasonable to assume that with the alarming rise in obesity worldwide, a MS epidemic may also be upon us. The prevalence of MS can alter depending upon the diagnostic criteria used, and has been reported to be as high as 50% in adults aged 60 years and over in the latest data from NHANES in 2009[137]. Within the United Kingdom, approximately 12 million adult men and women fulfil the diagnostic criteria of MS suggesting that prevalence is approximately 20-25% of the adult UK population.

United States

Most of the information on the prevalence of MS is sourced from the American National Health and Nutrition Examination Surveys (NHANES). Ford et al estimated that approximately 50 million Americans fulfilled the criteria for MS in 1990 with numbers rising to 64 million by 2000[160]. Two factors appeared to account for this increase. The first being obesity, which had risen from 22.5% to 30.5%. The second was an aging population, as the data revealed an increasing prevalence of MS with rising age groups regardless of BMI[17;137]. Hispanics being more prone to insulin resistance had a much higher incidence of MS as compared to other ethnic groups in the USA including Caucasians and Blacks. MS was more prevalent among Black and Hispanic females as compared to their male counterpart[160], an observation which contrasts with findings noted among Caucasians where the prevalence was higher among males. Black men particularly had a relatively low prevalence when compared to other ethnic groups, which is believed to be due to their lower waist circumference, lower triglycerides, and higher HDL-cholesterol levels. Black men are known to be more insulin-resistant and prone to hypertension although their other characteristics may potentially underestimate the prevalence of MS among them. The discrepancies have resulted in some suggesting that alternative parameters for the identification of MS within the Black African sub-division be used, although such alterations may no longer identify those with MS but those with high CV risk[204].

Of particular concern is the rising prevalence of the MS among the younger generation which has been attributed to the increase in obesity. NHANES, from data collected between 1988 and 1994, estimated the prevalence among adults aged 20

years as 24% with 7% of the overweight and 29% of obese adolescents fulfilling the criteria. A more recent study has shown that the prevalence has risen with 39% of the moderately obese adolescents, and 50% of the severely obese fulfilling the criteria. In total numbers this equates to approximately 1 million (4%) of all US adolescents[107;458;567].

Europe

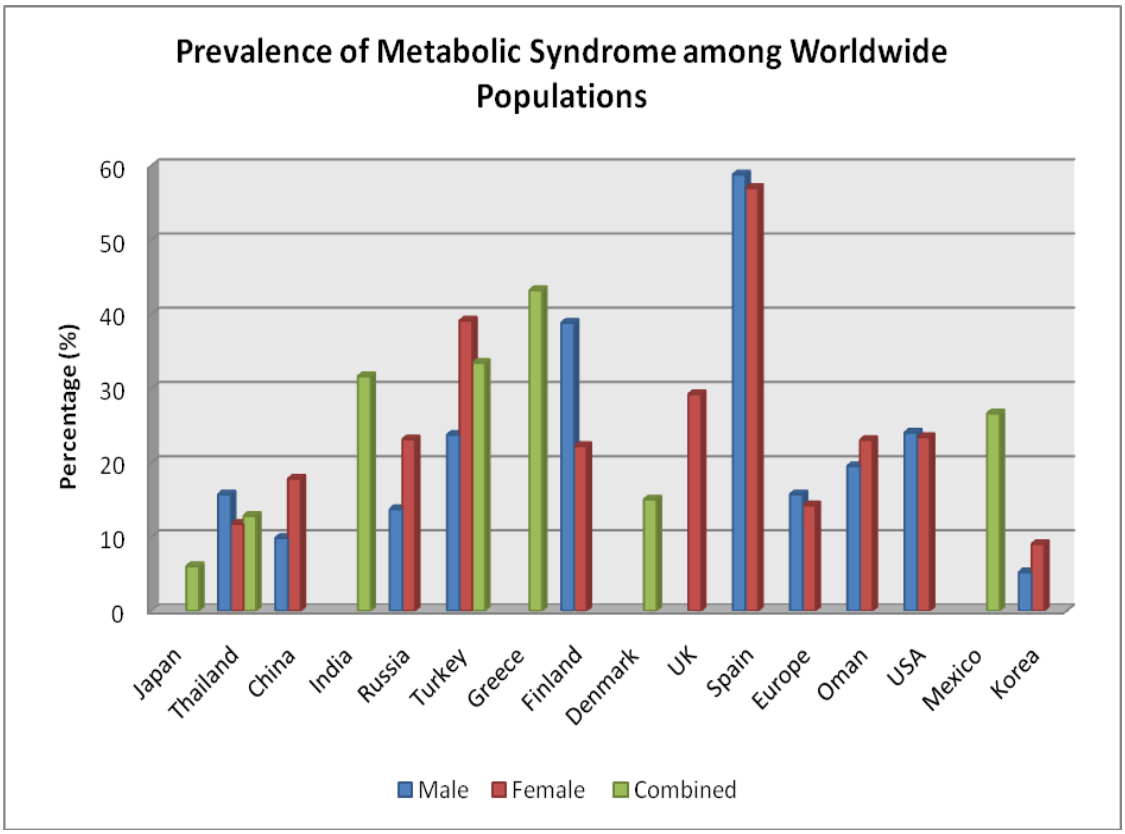
A series of studies on the occurrence of the MS in Europe have been reported. The prevalence has varied depending upon which criteria have been applied to define MS. The data suggest that approximately 25% of Europeans meet the criteria for MS with prevalence varying depending on the age group, nationality, and population characteristics. The prevalence was usually higher if IDF (50%) criteria were used as compared to NCEP (17.1%) or WHO (34.1%). These differences were attributed to the lower waist circumference threshold used to define abdominal obesity[65;337].

Worldwide

As with attempts in assessing the prevalence of MS among the European population, results for worldwide populations are variable. There are several variations depending on classification used, ethnicity, populations, and age groups. Among Turkish and Indian populations, the prevalence of MS has been estimated to be greater than 30%, whereas figures from Far Eastern populations were as low as 5%. As with Hispanics and Blacks, female prevalence of MS was higher among those from an Eastern origin than among Caucasians. Socioeconomically, those in urban communities were more likely to fulfil the criteria for MS, particularly in developing countries where rural life is physically intense and nutrition more basic.

One thing that was clear was that the prevalence of MS is increasing among all populations (Figure 7). The presence is higher in the older age groups but is also rising among the younger generations who are now becoming more obese and developing diseases which have previously been considered to be exclusive to adults.

Figure 6: Prevalence of Metabolic Syndrome Worldwide



Adapted from[134;160;265;269;291;404;493]. – Greek numbers based on >70yrs and Spanish >50yrs population group.

1.3.4. Abnormalities associated with Obesity and Metabolic Syndrome

Dyslipidaemia, hypertension, insulin resistance, and inflammatory markers are well-recognized predictors of CVD risk. Raised total cholesterol and LDL-cholesterol, as well as reduced HDL-cholesterol level, are associated with the risk for CHD. An elevated BMI is well recognised to be linked to an increase in cardiovascular disease with visceral adiposity identified as a strong independent predictor of insulin resistance and cardiovascular risk. Visceral adiposity is responsible for the increased abdominal circumference and the "apple" shape which is linked to an enhanced cardiovascular risk[34;370]. The majority of these abnormalities are seen in overweight, insulin-resistant individuals (table 7), while some will be discussed in further detail.

Table 7: Metabolic changes linked to the Metabolic Syndrome

Abnormalities associated with the metabolic syndrome
Visceral obesity
Hypertension
Dyslipidaemia
Hypertriglyceridaemia
Reduced HDL-cholesterol
Small dense LDL-cholesterol
Insulin resistance
Impaired glucose tolerance
Impaired fasting glycaemia
Type 2 diabetes mellitus
Hyperuricaemia
Microalbuminuria
Prothrombotic state
Raised fibrinogen
Raised PAI-1
Increased platelet aggregation
Proinflammatory state
Raised CRP
Raised inflammatory cytokines
Low adiponectin
Polycystic Ovary Syndrome
Obstructive Sleep Apnoea

1.4. Cardiovascular Disease

In 1987, Reaven suggested that insulin resistance played a central role in the MS. Several studies went on to strengthen this proposition with some implying that although insulin resistance may be the central component, other factors (i.e. genetic predisposition, obesity, dyslipidaemia, hypertension and physical inactivity), contribute to the cluster that formulates the metabolic syndrome[205]. It is difficult to assess the degree of influence each factor plays in the cardiovascular risk associated with MS, although it is undeniable that their influence enhances the risk of CV disease by as much as two to threefold as witnessed by the presence of diabetes[304](Figure 7).

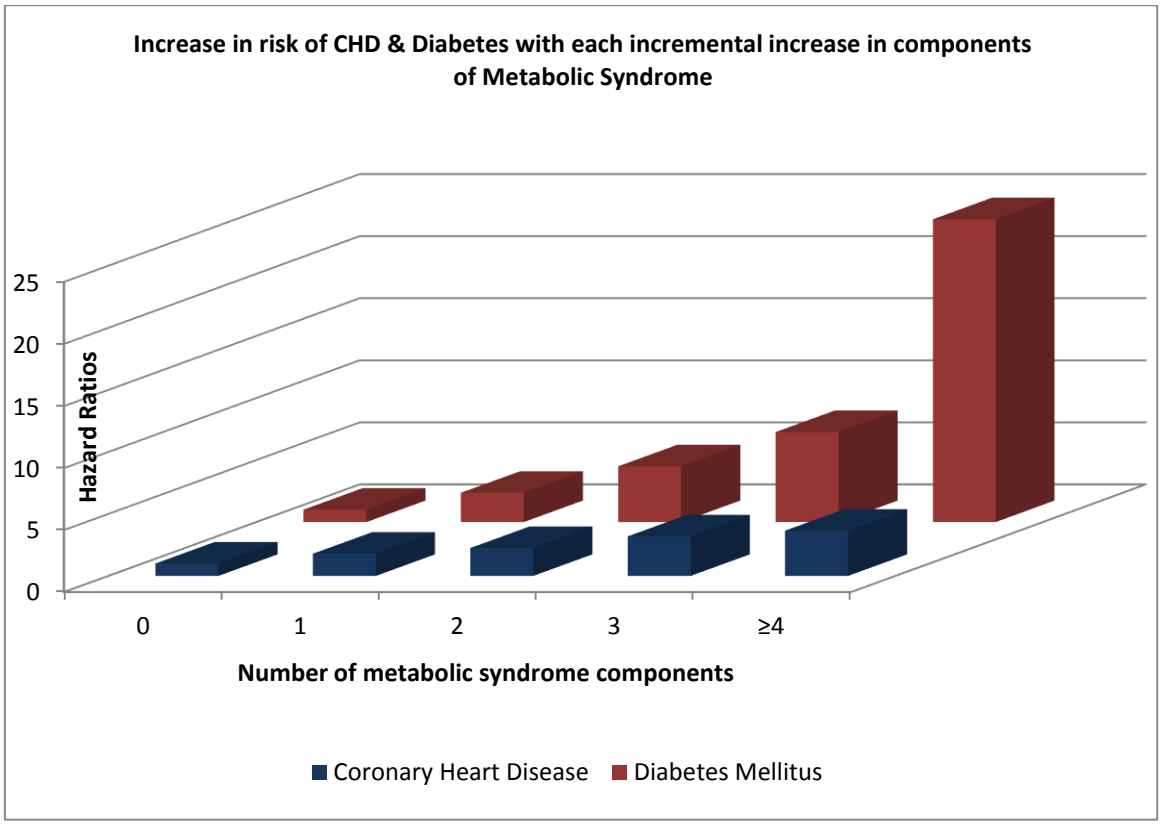
Over the past 30 years LDL-cholesterol has been targeted as an essential factor in CHD and numerous studies have highlighted the importance of LDL-cholesterol reduction on cardiovascular morbidity and mortality. Statins have certainly proven to be effective in modifying these risks[32;414;481]. The rising prevalence of MS and its associated increase in CV risk could potentially reverse the benefits achieved from LDL-cholesterol lowering and place MS on a par with smoking as a contributor to CHD[26;98].

Analysis of NHANES III as compared to NHANES II, demonstrated a rising prevalence of MS with worsening glucose tolerance with the presence among individuals with diabetes being very high (86%). Indeed, when the data were analysed for CHD, it was the presence of MS rather than diabetes that influenced CV risk Prevalence was highest within the group with both diabetes and MS (19.2%) or MS only (13.9%), while for diabetes only (7.5%), the presence of CHD was equivalent to, if not less than, that in the general population who had neither diabetes or MS (8.7%). The data did not differentiate Type 1 from Type 2 diabetes, and the authors concluded that it may be insulin resistance rather than hyperglycaemia which affected CHD risk[18]. In the West of Scotland Coronary Prevention Study (WOSCOPS) Sattar el al, defined MS by using BMI readings rather than waist measurement. Participants were stratified depending on the number of criteria they fulfilled for MS and followed through to assess for the risk of developing CHD or diabetes. Results confirmed that the more components for MS present, the greater was the risk for developing either

condition reaching 3.7 fold for CHD and 24 fold for diabetes when a full house as defined by NCEP ATP III was present[471] (Figure 7).

These findings were echoed in the Botnia study where 4483 Finnish and Swedish subjects aged 35 to 70 years were assessed. In addition to the incremental increase in MS prevalence with worsening glucose intolerance, its presence conferred a significant increase in the risk of CHD, stroke, and cardiovascular mortality[304].

Figure 7: Rising incidence of CHD and Diabetes with Metabolic Syndrome

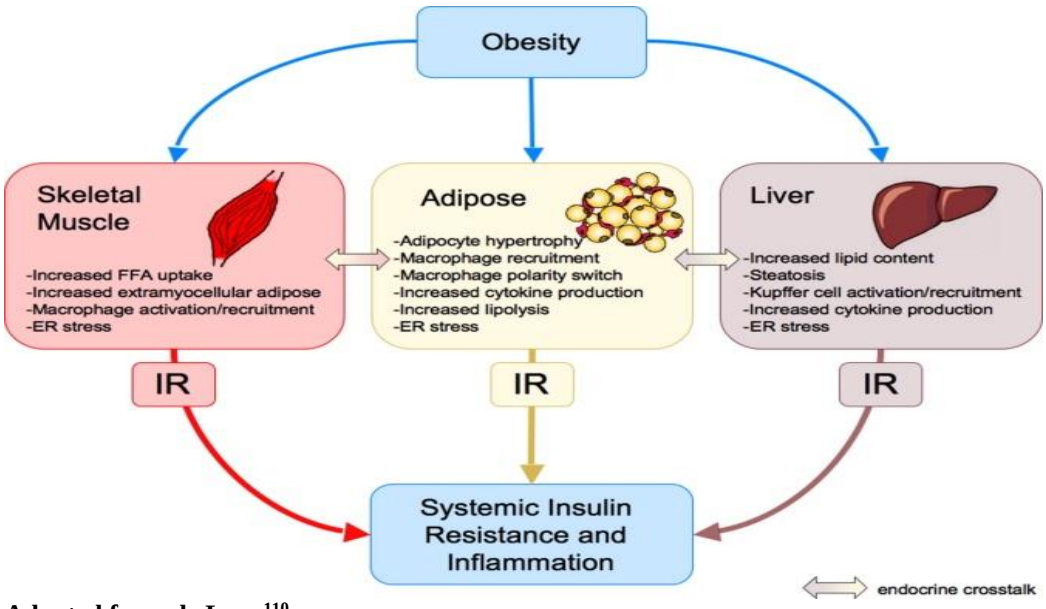


West of Scotland Coronary Prevention Study (WOSCOPS) – adapted from Sattar[471]

1.5. Insulin Resistance and Type 2 Diabetes Mellitus

Insulin has pleiotropic actions including the facilitation of nutrient transport into cells, modification of enzymatic activity and regulation of energy homeostasis (Figure 8). Insulin sensitivity refers to the ability of insulin to support glucose homeostasis by signalling insulin-sensitive tissues or organs to absorb glucose. These signals include stimulating glucose utilization in both muscle and adipose tissue and suppressing the production of glucose by the liver. Both responses act to decrease plasma glucose concentration. The degree of impairment of glucose metabolism is influenced both by the insulin sensitivity of cells within the body and by pancreatic β-cell reserve capacity[369].

Figure 8: Link between obesity, insulin resistance and inflammation



Adapted from de Luca[110].

Insulin resistance by definition is a condition in which defects in the action of insulin are such that normal levels of insulin do not sufficiently trigger the signal for glucose absorption resulting in the incomplete suppression of hepatic glucose output and impairment in insulin-mediated glucose uptake by peripheral organs such as skeletal muscle and adipose tissue, leading to hyperinsulinaemia. With time,

increased insulin requirements are not matched by a sufficiently increased insulin output leading to impaired glucose tolerance and eventually diabetes. Insulin resistance is associated with central obesity, hypertension, and dyslipidaemia, all well recognised risk factors for cardiovascular disease and components of MS[273;510].

The link between obesity and insulin resistance is strong with several studies indicating that obesity can account for ≥50% of the variance in insulin sensitivity in the general population. In a study of >250,000 subjects studying the relative risks for ischaemic heart disease and stroke mortality, a direct link relationship between fasting glucose levels and CV events was identified. For each 1mmol/l reduction in fasting glucose levels, there was a 21% coronary heart disease and 23% stroke mortality fall. No similar correlation was identified for prandial/random glucose levels. Other studies have demonstrated similar relationships, some with more modest results[455;470].

A major contributor to the development of insulin resistance is an excess of circulating fatty acids. These are derived mainly from adipose tissue triglyceride stores by the action of hormone sensitive lipase, or the lipoprotein lipase mediated lipolysis of triglyceride-rich lipoproteins in tissues. Insulin inhibits lipolysis within adipose tissue which, in the presence of insulin resistance, leads to accelerated release of NEFA and glycerol from adipose tissue, which further inhibits the anti-lipolytic effect of insulin[134]. The increased fatty acid load results in modifications in various transport signals such as impairing the activation of protein kinase C-λ and protein kinase C-ζ which further enhance insulin resistance[134].

Most hyperinsulinaemic states seen in MS do not convert to frank diabetes but once decompensation occurs and the balance is broken hyperglycaemia sets in along with all the associated complications known to diabetes (i.e. retinopathy, nephropathy, neuropathy and CVD).

1.6. Dyslipidaemia

Metabolic dyslipidaemia is widely recognised to be a risk factor for CV disease[72;73]. It consists of a combination of abnormalities which include hypertriglyceridaemia, an increase in VLDL, an increase in small dense LDL-cholesterol, and a reduction in HDL-cholesterol with abnormal HDL particles. Shortly following feeding, triglycerides and cholesterol are transported from the intestines to peripheral tissue in chylomicrons where triglycerides are hydrolysed via lipoprotein lipase (LPL) to release fatty acids which are taken up by muscle and oxidized for energy, or stored within adipose tissue. LPL is activated by apolipoprotein (apo) CII and inhibited by apoCIII. The chylomicron remnant is removed by the liver.

The liver synthesises triglycerides from lipids derived from chylomicron remnants, excess non-esterified fatty acids derived from adipose tissue, other nutrients including glucose, alcohol, excess amino acids, and intermediate metabolites. With these triglycerides are synthesised and secreted as very low density lipoprotein (VLDL) containing a single copy of apoB100 acting as both a structural component but also a functional moiety. VLDL particles have various other apolipoproteins which influence the particle metabolism.

In a manner similar to chylomicron metabolism, LPL hydrolyses VLDL-triglycerides for fatty acid use in peripheral tissues. Although some of the VLDL remnants can be removed by the liver, most are converted to low density lipoprotein (LDL) which delivers cholesterol to body tissues.

LDL accounts for the majority of measured serum cholesterol. Through the single apo B_{100} an LDL particle binds to a LDL receptor and is taken up into a cell. Cells up or down-regulate their LDL-receptors related to cell cholesterol content. The liver removes circulating LDL molecules through their own LDL receptors. Circulating LDL particles can vary in size as well as density with evidence demonstrating that the smaller denser particles being more atherogenic than their larger more buoyant counter-parts[99]. Excess circulating LDL concentrations are the main lipid particle responsible for cardiovascular disease risk, and many studies have shown that lowering serum LDL-cholesterol reduces cardiovascular events[26;27]. The

ratio of cholesterol to HDL-cholesterol is currently used (with other major cardiovascular risk factors) to estimate cardiovascular risk. However apoB100 may be a more predictive measure as it includes VLDL and VLDL remnant particles which can also be atherogenic, although non-HDL-cholesterol may be a more practical measure of the total of these particles, calculated from current non-fasting measurements[2;5;305;563].

HDL is the smallest of lipoproteins and composed of a core of apo A-I or A-II and phospholipids. It is synthesized by the liver and in gut, and participates in a reverse cholesterol transport pathway, able to accept cholesterol from cholesterol-replete cells for recycling. HDL appears to be associated with a number of activities, including anti-thrombotic, anti-oxidative, anti-inflammatory roles etc. all of which potentially facilitate its activity in protecting against atherosclerosis [54;540].

Other apolipoproteins associated with these lipoprotein particles modulate their metabolism. Enzymes such as acyl-cholesterol acyl transferase (ACAT) and cholesterol ester transfer protein (CETP) are involved in the transfers of triglycerides, cholesterol and cholesterol ester between cells and lipoproteins, and between different lipoproteins.

In normal circumstances the above interact harmoniously but can be dysregulated in MS and obesity producing dyslipidaemic features. Insulin resistance is believed to be central to this dysregulation. As an anabolic hormone insulin affects lipid metabolism through

- Inhibiting hormone sensitive lipase
- Reducing fatty acid release from adipose tissue
- Inhibiting hepatic gluconeogenesis
- Inhibiting hepatic glycolysis
- Increasing VLDL production through increased fatty acid and triglyceride production
- Encouraging adipose tissue fatty acid uptake through stimulating LPL activity

In the presence of insulin resistance there is:

- Enhanced adipose tissue lipolysis due to a relative intracellular insulin deficiency and the unopposed activity of the catabolic hormones, catecholamines and cortisol, with an increase in circulating NEFAs and hypertriglyceridaemia
- Enhanced production of apo B
- An increase in triglyceride-rich VLDL
- Inhibition of LPL activity
- Formation of small dense LDL particles[135]

The above metabolic effects produce a cascade which drives hepatic over-production of triglyceride-rich VLDL. The circulating VLDL through CETP mediation exchanges its triglycerides for cholesterol esters from both LDL and HDL leading to those particles being cholesterol-deplete but triglyceride-rich[260;405]. These LDL particles are rapidly hydrolysed into a small dense atherogenic format which is more susceptible to oxidation, resistant to clearance via LDL receptors, and has an increased affinity for vascular endothelial lining[42;90]. The LDL particle size is linked to triglyceride levels with an increasing predominance of smaller denser particles as triglyceride levels rise. In fact some studies have suggested that atherogenic LDL begins appearing at triglyceride levels >1.5mmol/L[197;211]. A similar association was noted in the Framingham Offspring Study where LDL particle size appeared to be inversely related to the number of MS components[275]. Measured LDL-cholesterol levels in these individuals are usually normal although the number of LDL particles is very likely to be increased. These inconsistencies have led to a dilemma as to how best to measure atherogenicity, should this be by counting particle numbers and therefore measuring LDL apo B levels (ratio 1:1 per LDL particle) or should it be through measuring total apo B (non HDL-cholesterol) levels which include VLDL and IDL-cholesterol in addition to LDL-cholesterol[198].

As with LDL, HDL particles in insulin resistance are low in cholesterol but full of triglycerides and therefore a target for hydrolysis. This causes an imbalance within particle components, destabilization of the complex, with enhanced removal of apo A, the core component making up HDL. Apo A is then renally excreted reducing circulating numbers of HDL as well as its protective measures[290].

1.7. Hypertension

Hypertension is commonly seen in adult individuals and is a well-recognised risk factor for CVD. Usually asymptomatic, it tends to be diagnosed during routine medical assessments and can be poorly controlled due to a combination of patient and physician factors. Until 1997 blood pressure targets were 160/ 90mmHg, then lowered to 140/90mmHg following the publication of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) in 2003 (Table 8). These guidelines were further supported by similar recommendations in the UK by the British Hypertension Society (BHS IV) (Table 9), and then in Europe by the European Society of Hypertension guidelines[194;572].

Table 8: Joint National Committee blood pressure targets

	Systolic	**Diastolic**
Normal	<120	<80
Prehypertension	121 - 140	81 - 90
Hypertension		
Stage 1	140 - 159	90 - 99
Stage 2	>160	>100

Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) Blood Pressure Targets adapted from Chobanian [95].

Table 9: British Hypertension Society Hypertension Targets

	Systolic	**Diastolic**
Low Normal	<120	<80
Normal	121 - 129	80 -84
High Normal	130 - 139	84 -89
Hypertension		
Grade 1	140 - 159	90 -99
Grade 2	160 -179	100 - 109
Grade 3	>180	>110

British Hypertension Societies (BHS) and European Hypertension Targets adapted from Williams and Graham[194;572].

The prevalence of hypertension defined as a blood pressure >140/90 is approximately 27.5% among North Americans, and 44% in Europeans including the UK[577]. Hypertension is a multifactorial disorder with numerous aspects including genetics, ethnicity, weight, family history, diet, stress, activity, hormonal dysregulation, personality traits, and drugs playing variable roles[586]. Obesity and insulin resistance are particularly believed to be major contributors in its evolution with several studies describing the direct relationship between fasting glucose levels, insulin resistance and obesity to hypertension[121;151;488;499]. Controversy exists as to whether insulin resistance results in hypertension or vice versa. Those supporting the theory that insulin resistance is causal for hypertension believe this is through stimulating sympathetic drive, arterial wall hypertrophy and resistance, and renal sodium retention[11;116;170].

It is well documented that hypertension increases mortality through its effect on cardiac disease, stroke (both haemorrhagic and ischaemic), and renal dysfunction. From meta-analysis of results of randomised controlled trials each lowering of systolic BP by 20mmHg halves the cardiovascular risk with no identified lower limit. Some accept similar reductions with a 10mmHg reduction in diastolic pressure, although others believe that it is the systolic level which is the dominant risk[431;431].

As a component of MS, the CV risks associated with hypertension are further enhanced with the presence of each additional component[474]. In diabetes, the presence of hypertension is common with a prevalence of greater than 50%[36]. Many studies have identified the added benefits of treating hypertension in this group of individuals[1;412;598].

In the United Kingdom Prospective Diabetes Study (UKPDS) 1148 hypertensive individuals with type 2 diabetes were found to benefit from improved blood pressure control on captopril or atenolol with reductions in microvascular outcomes of 37% for microvascular endpoints, 32% for diabetes-related deaths, and 44% for stroke. No significant improvement in the rate of myocardial infarct event was demonstrated. It is worth noting that at the time the average blood pressure readings in the intensive group was 144/82mmHg and 154/87 for the standard making the reductions -10/-5mmHg[20]. The Hypertension Optimal Treatment (HOT) trial included 1501 individuals with diabetes and hypertension (diastolic 100-115mmHg)

who were randomised aiming to achieve diastolic blood pressure readings of <90, <85 and <80mmHg. CV events were reduced by 50%, CV mortality by 65% and although not significant, myocardial infarction by 50% in the group who achieved a reading of <80 compared to <90[217]. Following the publication of these trials blood pressure targets in individuals with diabetes were reduced from <140/90 to <140/80 and to <130/80 in more recent years[436;572].

Obesity's effect on blood pressure is multi-factorial as this may be partly through a direct impact or indirectly through its association with insulin resistance, and possible fluid retention. Certainly several trials have demonstrated a reduction in blood pressure with weight loss. A meta-analysis of 25 weight loss studies reported that an average weight loss of 5kg reduced systolic blood pressure by 6.5mmHg and diastolic by 5mmHg in individuals who were not on any hypertension medication. Bigger results were noted in those on treatment for hypertension[386]. In the Trials of Hypertension Prevention, approximately 1200 overweight adults with high normal or prehypertension, were randomised to weight loss, sodium intake reduction, combined weight loss and sodium reduction, or a control group and followed for 3 years. Blood pressure was documented at 6, 18 and 36 months. The target for the weight loss group was to lose 4.5kg at six months and maintain this throughout the trial. They achieved their initial weight loss and at six months the SBP/DBP readings were down by 3.7/2.7mmHg in the weight loss arm, 2.9/1.6mmHg in the low salt arm and 4/2.8mmHg in the combined group. The weight was gradually regained by the end of the study but despite this, all interventions managed to maintain meaningful reductions in blood pressure[517].

1.8. Inflammatory State

An inflammatory response is a series of complex pathways which are activated in response to an irritant, be it pathogen or foreign body, by which the body attempts to self-heal. The processes involved usually include the activation of the innate immune system with subsequent release of pro-inflammatory cytokines. In acute or chronic clinical inflammatory conditions (i.e. rheumatoid arthritis, inflammatory bowel disease) levels of circulating inflammatory markers are high and have been associated with an increased CV risk[120]. In obesity and MS, the same inflammatory markers have been identified as being elevated, although not as high as seen in well documented chronic inflammatory conditions. It is understood that these small elevations are sufficient to bring about changes that predispose to atherogenesis and CV disease over longer periods of time.

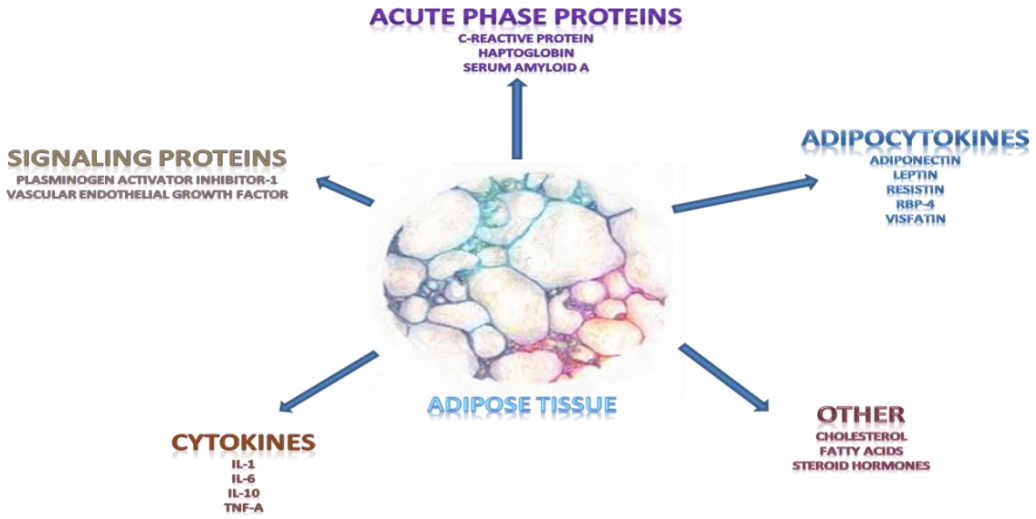
It has been well documented that adipose tissue is not an inert storage facility but actually a dynamic, metabolically active organ which stores free fatty acids and regulates their release back into the circulation when required for energy. It also secretes its own biologically active molecules, known collectively as adipocytokines, in addition to chemokines and inflammatory proteinoids which individually or collectively interact with various biological processes that suppress or enhance inflammation, immunity or prothrombotic tendencies[342;353;491;537] (Figure 9).

In 1840 J. Paget considered fat to have a number of functions including, "1) a giver of lightness, especially to bones, 2) to spread local pressure over large areas by using adipose tissue as an incompressible elastic substance (i.e. soles of feet, pressure area), 3) to serve as a passive material that fills up spaces between adjacent organs whose functions require them to possess peculiar forms (i.e. heart, eyeballs, gut), 4) a protective against the cold, 5) to give roundness of form with which (among other advantages) the limbs and trunk meet the least resistance when traversing the air or water, and 6) as a disordered secretion"[408].

Many studies have demonstrated the association between circulating inflammatory markers such as C-reactive protein, IL-6, resistin, PAI-1, and tumour-

necrosis factor, with the components of MS. In addition adiponectin secretion, an adipocytokine, identified to enhance insulin sensitivity, and have anti-inflammatory properties is suppressed in the presence of obesity. How it exerts its effects is unclear, but its deficiency is coupled with insulin resistance, pro-thrombotic tendencies and inflammation.

Figure 9: Adipokines secreted by adipose tissue



The precise mechanism by which an inflammatory response is initiated in obesity or MS is not defined. Abnormal visceral fat accumulation is associated with inflammatory changes, including recruitment of macrophages and activation of endothelial cells, which promote vascular disease[277]. Some believe that as adipose tissue increases in mass, it outstrips its blood supply, resulting in intracellular hypoxia and cell apoptosis which releases pro-inflammatory cytokines and attracts macrophages. Indeed, several molecules produced by adipocytes (adipocytokines) are associated with increased cardiovascular risk partly through increased expression of pro-inflammatory genes and induction of systemic inflammation[110;595]. A reduction in the secretion of inflammatory molecules can be achieved through weight loss, implicating adipose tissue mass as a partial regulator of inflammation.

An alternative pathway is that the accumulation of adipose tissue leads to increased oxidative stress partly via the oxidant effects of fatty acids. Leukocytes

derived from obese individuals infused with free fatty acids suffer from increased oxidative stress. Oxidative stress in turn may promote metabolic complications such as insulin resistance and induce a pro-inflammatory state via deregulation of adipocytokines[176]. Thus, oxidative stress is both induced by and adversely impacts on adipose tissue function. Consequently, biomarkers for oxidative stress may play a role in obesity-induced CVD. Enhanced levels of oxidative stress are linked to smoking, hyperglycaemia, dyslipidaemia and obesity[84;152;277]. In animal studies a rise in vascular cell adhesion molecule-1 (a molecule which promotes the adhesion of monocytes and lymphocytes to the intimal surface of the endothelium) was noted following a short term atherogenic diet providing evidence that factors apart from traditional ones may affect atherosclerosis[34;323].

Although insulin resistance is believed to be central to the low grade inflammation seen in MS and obesity, a "chicken or egg" situation appears to exist. Insulin as an anabolic hormone regulates the uptake and storage of glucose and fatty acids in liver, muscle and adipose tissue. In the presence of insulin resistance, there is hyperglycaemia, enhanced lipolysis and an abundance of circulating NEFA. NEFA induce oxidative stress, an inflammatory response and through blocking various transduction signals insulin resistance and thus proceeds a vicious cycle[541].

1.9. Management Recommendations for Metabolic Syndrome

The association between obesity and the development of MS constitutes the main argument justifying weight reduction programmes which, if successful, can lead to a substantial improvement or normalisation of the metabolic profile of the obese[373;379;538;573]. A 10kg reduction in weight equates to a 20% fall in total morbidity, 15% fall in LDL-cholesterol (LDL-C), 30% fall in triglycerides and an 8% rise in HDL-cholesterol (HDL-C)[479]. A 15-20% weight loss in the first year after diagnosis of type 2 diabetes may reverse the excess mortality of being overweight and a deliberate weight loss of 5-9 kg is associated with up to a 30-40% reduction in diabetes-related mortality[39].

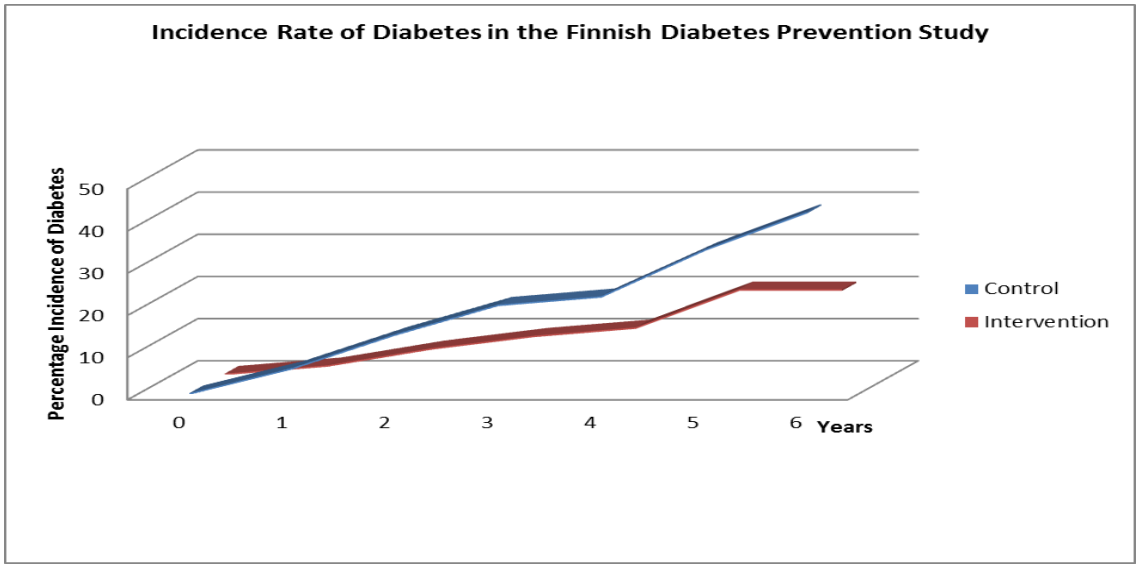
Huge numbers of individuals in the developed world profess to be "on a diet" at any given time. In the USA alone approximately 35% of women and 45% of men aim to lose weight, but despite their efforts, the prevalence of obesity has doubled in the past 20 years[164] suggesting that the given dietary advice may itself be a contributing factor. The conventional dietary approach to weight management recommended by the leading research and medical societies[164] is a low fat, high carbohydrate, energy-deficient diet (30% energy from fat, 10-15% protein, 55-60% carbohydrate), but despite Americans consuming fewer fat-derived calories (42% in the 1960s and 33% in the 1990s[338;584]) the prevalence of obesity increased[60]. Consequently high carbohydrate diets, particularly those containing high glycaemic index foods and low fibre content, are now proving to be controversial as epidemiological, clinical and experimental studies[312] indicate that such diets may in fact contribute to weight gain as they increase circulating plasma triglycerides, thus affecting LDL composition, reducing oxidation of body fat and reducing satiety[312;486].

There are two general approaches to address the management of MS. The first of these targets the root causes, 'increased BMI and physical inactivity', which have a knock on effect on the closely linked insulin resistance. Weight reduction and increased physical activity both lower insulin resistance and indirectly favourably affect the metabolic risk factors. The second approach directly treats the "metabolic" risk factors of atherogenic dyslipidaemia, hypertension, prothrombotic state, and underlying insulin resistance through the use of medications.

1.9.1. Lifestyle Changes and Weight-Loss:

Addressing obesity and physical inactivity will reverse the fundamental cause of MS and should be the first line of intervention in any cardiovascular risk reduction programme. Data from the NHANES II demonstrated that an elevated BMI was associated with elevated triglycerides and low HDL-cholesterol. NHANES III displayed the link between elevated arterial blood pressure and variations in body weight. A number of studies have shown that weight loss and increased physical activity will delay or prevent the onset of type 2 diabetes in individuals with the metabolic syndrome. The Finnish Diabetes Prevention Study randomised 522 non-diabetic, middle-aged, over-weight individuals with impaired glucose tolerance to either a lifestyle intervention or a control group. The intervention goals included a targeted 5% weight reduction, reduced dietary fat intake, and at least 30 minutes of aerobic exercise daily. Subjects were followed for a mean of 3.2 years, with annual glucose testing.

Figure 10: Incidence of Diabetes in Finnish Diabetes Prevention Study



Adapted from Tuomilehto [543]

The risk of diabetes was reduced by 58% in the intervention group, with the reduction being directly associated with the lifestyle changes (Figure 10). The improvements were sustained in those who achieved their target intervention goals with a relative risk reduction at 7 years equivalent to 36% (incidence rate of 4.6% in the intervention group and 7.2% for the controls) [328;329;543].

The Diabetes Prevention Program Research Group randomised 3234 non-diabetic subjects with elevated fasting and post-prandial glucose levels to placebo, lifestyle modification, or metformin (850 mg twice daily). Targets for the lifestyle group included 7% total body weight loss and two and half hours of weekly exercise. Individuals were followed for approximately 2.8 years. Reductions in incidence of diabetes were similar to the Finnish Diabetes Prevention Study with the lifestyle group achieving a 58% reduction in incident diabetes when compared to the placebo group, with a 39% reduction in the metformin group[76]. In the Nurse's Health Study 84,000 healthy females participated and were followed for more than 14 years. Those following a healthy lifestyle (i.e. non-smokers, BMI < 25kg/m2, regular exercise and low alcohol consumption) were reported to have a relative risk of coronary events of 0.17 as compared to all other women[509].

Thus with the evidence clearly favouring the need for lifestyle modifications, NCEP recommended that weight loss be the first target in those individuals with central adiposity and MS and set a target of 7-10% reduction in total weight within the first 6-12months. To achieve these targets it was advised to reduce caloric intake, and increase physical activity with recommendations being a minimum of 30 minutes of intensive activity on most days of the week. Weight loss could be assisted with the use of therapeutic agents, although those available have only had modest success. For severe obesity bariatric surgery could be considered[134].

Once weight loss has been achieved, on-going weight maintenance is just as important and requires a lot of support both emotionally and physically, to encourage the individual to remain motivated and persist with the healthier lifestyle choices. From results in the Counterweight programme, although no correlation was seen between weight loss and regain, individuals who lost a greater percentage of their weight (>10%) were less likely to fully regain it[2;292;314].

1.9.2. Physical Activity

Physical activity assists weight reduction, and has favourable effects on cardiovascular risk factors. Several physical activity studies have demonstrated the improvement in a number of CV risk factors (i.e. reduction in blood pressure and triglycerides and increase in HDL-cholesterol), and consequent reduction in cardiovascular events. In addition other benefits include improvements in myocardial function, cardiac perfusion, and vascular tone. Some studies have reported the incidence of coronary disease to be 50% lower in active groups as compared to their sedentary counterparts[529].

The current recommendations suggest 30 minutes of moderate-intensity exercise on most days, with extra exercise adding to the benefits. To achieve weight loss activity should be increased to sixty minutes of moderate aerobic exercise, supplemented by additional short bouts of high intensity exercise throughout the day. The American Heart Association recommends that those individuals with high cardiovascular risk should consult a specialist for a prescribed exercise programme to limit the risk of harm[224]. Despite the documented success of physical exercise in improving weight, insulin resistance and CV risk, compliance in reality is a recurring stumbling block. In an American health survey Kruger et al. identified that approximately one in three adults who participated reported successfully losing and maintaining their weight loss. These successful candidates were more health conscious, planned their meals, calculated caloric intake and engaged in regular exercise both aerobic and weight-bearing. On reviewing the obstacles to exercising regularly, these included lack of time, motivation, lack of an exercising partner, funding, and tiredness[296].

1.9.3. Dietary Modifications

The current recommendations state that the diet should not only be low in calories but low in saturated fats, *trans* fats, cholesterol, sodium, and simple sugars. People are expected to partake of ample portions of fruits, vegetables, whole grains and oily fish. Controversy remains around which diet should be promoted. ATP III advises that a diet for those with MS should help reverse the dyslipidaemia but any increases or decreases in dietary components will inevitably alter the percentage of the others and potentially affect the absolute quantities (i.e. a fat intake of greater than 35% potentially increases the saturated fat and caloric intake, while less than 25% may increase the carbohydrate portions). High carbohydrate intake, especially of refined carbohydrates can have a deleterious effect by encouraging hypertriglyceridaemia and reducing HDL-cholesterol.

There has long been an interest in the question of whether changing the macronutrient content of the diet can promote weight reduction. For many years, a low-fat diet was advocated because the high caloric density of fat could increase the likelihood of obesity. More recently, interest has grown in the possibility that high-protein, low-carbohydrate diets will enhance weight reduction. The rationale seems to be that fat and protein offer satiety that is absent with carbohydrates. That this effect of fat and protein on satiety makes the diet more effective for producing weight loss is a disputable hypothesis. Arguments arise that such diets lack essential recommended nutrients in the form of fruits, vegetables, and whole grains and cannot be tolerated by all individuals. In general successful weight loss requires a combination of caloric restriction, physical activity, and motivation; effective lifelong maintenance of weight loss essentially requires a balance between caloric intake and physical activity (Table 10)[201].

The "eatwell plate" visually describes how much an individual should eat from each food group including snacks (Figure 11). The general principal behind it is to encourage the consumption of[2]:

- plenty of fruit and vegetables
- plenty of bread, rice, potatoes, pasta and other starchy foods – choose wholegrain varieties when possible
- some milk and dairy foods
- some meat, fish, eggs, beans and other non-dairy sources of protein
- just a small amount of foods and drinks high in fat and/or sugar

Figure 11: The Eatwell Plate

People are advised to look at the eatwell plate and modify their food intake to something equivalent. They are expected to try to choose options that are lower in fat, salt and sugar when possible.

[2] The information on this page has been taken from the *eatwell.gov.uk* website[201].

Table 10: Lifestyle recommendations for cardiovascular risk prevention

Lifestyle intervention	Joint British Societies 2 Lifestyle Recommendations	American Guidelines for Lifestyle Management of Metabolic Syndrome
Smoking	Smoking cessation	Smoking cessation
		To maintain body weight in a healthy range, balance calories from foods and beverages with calories expended
Ideal Body Weight	Aim for BMI 20 -25kg/m2	Aim to reduce body weight by 7% to 10% during year 1 of therapy Then aim for BMI <25kg/m2
Waist Measurement	White Caucasians : <102 cm in men and <88 cm in women Asians: <90 cm in men and <80 cm in women	<102 cm in men and <88 cm in women
Physical Activity	Regular aerobic physical activity of at least 30 mins per day, most days of the week	To reduce CV risk: at least 30 minutes of moderate-intensity physical activity, above usual activity, at work or home on most days of the week. To sustain weight loss: at least 60 to 90 minutes of daily moderate-intensity physical activity while not exceeding caloric intake requirements
Dietary Modifications		
Fruit & vegetable	Fresh fruit and vegetables to at least five portions per day	Two cups of fruit and 2½ cups of vegetables per day
Grains		Consume 3 or more ounce-equivalents of whole-grain products per day, with the rest of the recommended grains coming from enriched or whole-grain products
Dairy Products		3 cups per day of fat-free or low-fat milk or equivalent milk products
Fat	Aim for dietary cholesterol <300 mg/day	Aim for dietary cholesterol <200 mg/dL;
	Aim for total fat ≤30% of total energy intake. Replace saturated fats by an increased intake of monounsaturated fats	Aim total fat intake between 20 to 35% of calories, with most fats coming from polyunsaturated and monounsaturated fatty acid.
	saturated fats to ≤10% of total fat intake	saturated fat <7% of total calories
	Regular intake of fish and other sources of omega 3 fatty acids	
Alcohol	Limit alcohol intake to <21 units/week for men or <14 units/week for women	
Salt	Limit the intake of salt to <100 mmol/l day (<6 g of sodium chloride or <2.4 g of sodium per day)	

Adapted from[26;200;579].

1.9.4. Targeting the Metabolic Risk Factors

The second approach to managing MS is to specifically target the different risk factors with the aid of pharmacological agents. Where a healthy lifestyle can be prescribed to the population in general, this alternative method is required for those who are at a high risk of CVD and would receive significant benefit. In the NCEP-ATP III recommendations individuals are stratified to low, moderate or high risk using the Framingham cardiovascular risk model (Table 11). They were automatically categorised as high risk if they already had diabetes or a known CV event.

Table 11: Framingham risk stratifications for establishing management targets

Risk level		**Hard CHD risk**
High	CHD or CHD Risk Equivalent	>20%
Moderate	≥2 Risk Factors*	10 – 20%
Low	≤1 Risk Factors*	<10%

The risk factors used here are cigarette smoking, hypertension (BP ≥140/90 mmHg or on treatment), low HDL-cholesterol (<40 mg/dl), family history of premature CHD and age[26].

1.9.4.1. Dyslipidaemia

Although MS is particularly identified with an atherogenic dyslipidaemia consisting of low HDL-cholesterol, small dense LDL-cholesterol and elevated triglycerides, guidelines recommend that it is essential to first address the well-established atherosclerotic risk presented by LDL-cholesterol. Only when treatment targets have been achieved and LDL-cholesterol has been reduced to 2mmol/l[3] or less should the focus then shift to addressing the other dyslipidaemic components. A large number of cardiovascular outcome studies have demonstrated the benefit of LDL-cholesterol lowering but similar favourable outcomes are yet to be demonstrated with agents targeting the other components of dyslipidaemia (i.e. fibrates, cholesteryl ester transfer protein inhibitors, and niacin)[53;100;101;115;141;189;454;478;481].

In 2005, The Joint British Societies guidelines were published and set targets for cholesterol management. These are stated as:

"to lower total cholesterol by 25% or LDL cholesterol by 30% or to reach< 4.0 mmol/l or < 2.0 mmol/l respectively, whichever is the greater.
However a total cholesterol concentration < 5.0 mmol/l or LDL cholesterol < 3.0 mmol/l or reductions of 25% or 30%, respectively (whichever is the greater), provides a minimal acceptable "audit" standard.
Also cardiovascular disease (CVD) risk replaces coronary heart disease (CHD) risk estimation to reflect the importance of stroke prevention as well as CHD prevention.
The new CVD risk threshold of ≥ 20% is equivalent to a CHD risk of approximately ≥15% over 10 years"[579] .

The National Institute for Health and Clinical Excellence (NICE) in 2010 published its updated version of the 2008 lipid management guidelines which recommended initial lifestyle and other risk factor modification for primary prevention in those with a >20% 10 year CVD risk and statin therapy for secondary prevention regardless of risk factor modification. Treatment targets in secondary

[3] Quality and Outcomes Framework (QOF) would set the target for primary prevention at <3mmol/l

prevention remain at <4mmol/l for total-cholesterol and <2mmol/l for LDL-cholesterol although there are ongoing discussions to potentially reduce LDL-cholesterol treatment targets to <1.8mmol/l particularly in high risk individuals (i.e. type 2 diabetes). Similar recommendations[26;261] are seen in other international lipid guidelines (Table 12) allowing for a stepwise approach in lipid management which may include:

1. Initial intervention with dietary and lifestyle changes
2. LDL-cholesterol lowering with statins with LDL-cholesterol target <3mmol/l in the low risk individual and <2mmol/l in the high risk individual. Diabetes and a previous cardiovascular event, would automatically be included in the high risk category.
3. Triglyceride lowering aiming to reduce the risk of pancreatitis in those with levels > 5.6mmol/L.
4. Increasing HDL-cholesterol to >1.3mmol/L although no benchmark target level has yet been identified.

Table 12: LDL Cholesterol targets for lifestyle and treatment

Risk Category	LDL Level at which to initiate lifestyle advice	LDL Level at Which to Consider Drug Therapy
High	≥100 mg/dL (2.6mmol/L)	≥130 mg/dL (3.4mmol/L) Drug therapy can be initiated at lower targets depending on physician's discretion
Medium	≥130 mg/dL (3.4mmol/L)	10-year risk 10-20%: ≥130 mg/dL (3.4mmol/L) 10-year risk <10%: ≥160 mg/dL (4mmol/L)
Low	≥160 mg/dL (4.2mmol/L)	≥190 mg/dL (5mmol/L)

Adapted from NCEP executive statement [26].

1.9.4.2. Hypertension

Blood pressure targets in people with MS are generally no different from those set for the general population. A target of <140/90mmHg is aimed for in the majority with levels falling to <130/80mmHg in individuals with diabetes (Table 13).

In those with mildly elevated readings, the importance of lifestyle changes are to be emphasised including dietary sodium reduction, moderation in alcohol, weight loss and increased activity, which are established as effective first line measures for blood pressure reduction. Recommendations were made to target antihypertensive therapy according to an individual's needs. Thus those with cardiac disease should preferably be initiated on beta-blockers and/or ACE-inhibitors whilst those with diabetes should receive ACE-inhibitors or ARBs for renal protection. It was acknowledged that in most situations a combination of agents would be required in order to achieve targets in the more complex patients[26;199;579].

Table 13: Target to treat to levels for parameters of the Metabolic Syndrome

Cardiovascular Parameter	**Joint British Societies 2 Lifestyle Recommendations**			**American Guidelines for Lifestyle Management of Metabolic Syndrome**		
	Persons with cardio-vascular disease	Persons at high risk (≥20%)	Persons with diabetes mellitus	Persons at low risk (<10%)	Persons at medium risk (10 -20%)	Persons at high risk (>20%) or diabetes mellitus
Blood pressure	<130 mm Hg systolic and <80 mm Hg diastolic	<140 mm Hg systolic and <85 mm Hg diastolic	<130 mm Hg systolic and <80 mm Hg diastolic	<140 mm Hg systolic and <85 mm Hg diastolic	<140 mm Hg systolic and <85 mm Hg diastolic	<130 mm Hg systolic and <80 mm Hg diastolic
Lipids						
Total Cholesterol	<4.0 mmol/l	<4.0 mmol/l	<4.0 mmol/l			
LDL-Cholesterol	<2.0 mmol/l	<2.0 mmol/l	<2.0 mmol/l	4mmol/L	<3.6mmol/L	<2.6mmol/L
Glucose						
Fasting Plasma Glucose	≤6.0 mmol/l	≤6.0 mmol/l	≤6.0 mmol/l			
			HbA1c <6.5%			HbA1c <7%
Proinflammatory and Prothrombotic state	Aspirin 75mg od	Aspirin 75mg od	Aspirin 75mg od*	Aspirin 75mg od	Aspirin 75mg od	Aspirin 75mg od *

* Aspirin is no longer recommended as primary prevention for diabetes unless there is an increased cardiovascular risk. Adapted from[59;200;421;579].

1.9.4.3. Hyperglycaemia

Lifestyle interventions are important to delay the progress to frank diabetes in those who are glucose intolerant, and to avoid microvascular and macrovascular morbidities in those with established type 2 diabetes. Should these measures fail, then the addition of hypoglycaemic agents is recommended for type 2 diabetes. First line agents have included metformin and sulphonylureas. Thiazolidinediones theoretically help improve insulin resistance and preserve pancreatic function but recent evidence questioned their benefit in cardiovascular risk reduction, safety in cardiac dysfunction, risk of bladder neoplasm and fractures, resulting in the withdrawal of rosiglitazone and limited use of pioglitazone [126;140;241;390]. Oral agents are not recommended for those without established diabetes although some tend to administer metformin in obese females with polycystic ovary syndrome. Targets are to achieve normoglycaemia in those with impaired fasting glycaemia and HbA1c of <6.5% in those with established diabetes on metformin (Table 13). Alternative targets are set for the vulnerable (at risk of hypoglycaemia particularly the elderly and those with disabilities), and those on insulin.

1.9.4.4. Hypercoaguable State

MS has been well documented to be a pro-coagulant state with increases in the level of fibrinogen, plasminogen activator inhibitor-1, and other coagulation factors which potentially accelerate arterial thrombosis. Current recommendations to counteract the potential risk of thrombosis in both primary and secondary prevention has been low dose anti-platelet agents in the form of aspirin or clopidogrel where aspirin is not tolerated. A recent trial has failed to demonstrate the benefit of aspirin for primary prevention in individuals with diabetes but highlighted the increased risk of bleeding. As a result recommendations have now changed and aspirin is only recommended in secondary prevention or in those with an increased risk of CV disease[59;421]. The ASCEND trial which is currently investigating the use of aspirin in diabetes for CV risk prevention has recruited 15,000 individuals with diabetes and is expected to report in 2017[35].

1.9.4.5. Proinflammatory State

As yet no actual drugs have been produced to target the pro-inflammatory state associated with MS. Weight reduction through lifestyle changes is currently the best recommendation. Many of the generally used cardiovascular drugs such as statins, ACE inhibitors, or pioglitazone have been reported to affect beneficially some of the various inflammatory markers but they are not recommended to be used purely as an anti-inflammatory agent[281;385]. In fact recognised anti-inflammatory agents have been associated with increased cardiovascular risk. Individuals taking them for prolonged periods should do so under supervision[74;163].

1.10. Drugs used in the treatment of Obesity

Living organisms obey the first law of thermodynamics, and their body weight depends ultimately upon the balance between energy intake and output. Consequently, the current available agents used in achieving weight loss work along one of three strategies, to stimulate anorexigenic signals, oppose orexigenic signals, or increase energy expenditure. Up until 2009 there were three drugs available to be used to target weight loss. These were orlistat, sibutramine, and rimonabant. Both Sibutramine and Rimonabant have had to be withdrawn due to adverse side-effects leaving Orlistat as the only contender. Glucagon like Peptide (GLP-1) agonists, have recently been released on the market for the management of type 2 diabetes mellitus. Their potential to reduce weight has made them possible agents particularly in the obese with high cardiovascular risk and type 2 diabetes.

1.10.1. Orlistat

Orlistat (Xenical) is a derivative of lipstatin which inhibits gastric and pancreatic lipase degradation of triglycerides within the gut, decreasing fat absorption, encouraging its elimination in the stool, and reducing the overall caloric intake. Studies have proven that it may reduce triglyceride absorption by up to 30%. Data from several trials have shown that regular use of this drug in its prescribed dosage of 120mg three times daily in the context of a low fat diet, produced an additional 5% weight loss above that sustained by caloric restriction. The weight loss was maintained for the initial two year study period[498;513;534]. Not only did orlistat help with weight loss but additional data demonstrated favourable changes in blood pressure, lipid profile, particularly post prandial hypertriglyceridaemia and glycaemic control[125;244].

In the XENDOS (XENical in the prevention of Diabetes in Obese Subjects) study, the use of orlistat in 3,300 obese subjects reduced the risk of progression to

diabetes by 37%, when compared to placebo[535]. The benefits were predominantly observed in those with IFG and IGT. In the Orlistat and Cardiovascular risk profile in patients with metabolic syndrome and type 2 Diabetes (ORliCARDIA) study, 126 individuals with type 2 diabetes on oral treatment, fulfilling the criteria for MS were randomised to a hypocaloric diet with orlistat or diet alone, and followed for 6 month. Significant favourable changes in weight, blood pressure, LDL-cholesterol, fasting glucose and insulin resistance were noted in the orlistat arm with the researchers concluding that it may have protective cardiovascular benefits[125]. Orlistat has also been shown to reduce C-reactive protein, and adipokines (interleukin-6 and *a*-tumour necrosis factor) as well as raise adiponectin levels[593]. The clinical impact of these findings is yet to be evaluated.

Despite its benefits, orlistat's use is limited by its side effects which are mainly gastrointestinal. The adverse effects, are not serious, but can be embarrassing. They include increased defecation, fatty/oily stools, leaking of oil from the rectum, faecal urgency and faecal incontinence. The symptoms can be avoided with proper counselling and maintaining a low fat diet, being related to higher fat meals. They tend to reduce with time. In addition there is a potential for a reduction in fat-soluble vitamins with reduced intestinal absorption leading to recommendations that individuals should take multi-vitamins to counteract the effect[71].

Up until now no long term cardiovascular outcome data are available for orlistat and this is surely required to assess its overall benefit, apart from weight loss, and define its place in cardiovascular risk prevention.

1.10.2. Sibutramine

Sibutramine (Reductil) is a β-phenethylamine that acts centrally selectively inhibiting the reuptake of noradrenaline, serotonin (5-HT) and, to a lesser extent, dopamine. It was originally developed as an anti-depressant but during experimental phases, it was noted to increase thermogenesis and suppress appetite thus reducing caloric intake. Trials confirmed that sibutramine could reduce weight by up to 9% which could increase to 15% when combined with caloric restriction. In a meta-analysis of 8 weight loss studies in type 2 diabetes sibutramine reduced weight, waist circumference and triglycerides, improved glycaemic control and raised HDL-cholesterol[559]. The meta-analysis confirmed that sibutramine increased systolic and diastolic blood pressure and heart rate which restricted its initial use. Other adverse symptoms reported included headaches, insomnia, dry mouth and constipation. Unlike its predecessors, fenfluramine, and dexfenfluramine, sibutramine was not associated with pulmonary hypertension, or valvular dysfunction[406;461].

In January 2010, following an interim analysis from the Sibutramine Cardiovascular Outcomes (SCOUT) Trial, the European Medicines Evaluation Agency (EMEA) and the Medicines and Healthcare products Regulatory Agency (MHRA) produced a statement withdrawing it use from European markets. At the same time the Federal Drugs Agency in the USA added cardiovascular disease as a contraindication for sibutramine.

SCOUT was a cardiovascular outcome trial which randomised over 10,000 obese patients with high cardiovascular risk to sibutramine or placebo, plus lifestyle changes. Individuals were to be followed for up to 5 years. Primary outcomes included the incidence of non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death. The interim analysis from SCOUT demonstrated that cardiovascular events were higher in the sibutramine arm (11.4%) as compared to placebo (10%). Although seemingly small, the difference was enough to pronounce that the risk of sibutramine far outweighed its potential benefits, especially as it had been linked to prolongation of the QT interval, cardiac arrhythmia and death[23;461]. Arguments arose that the individuals recruited to SCOUT would normally have been contra-indicated for the use of sibutramine, but with obesity being closely linked with cardiovascular morbidity, there was a potential for inappropriate use.

1.10.3. Rimonabant

Rimonabant (Accomplia) was a selective antagonist of the cannabinoid receptor CB1 exerting its effect through appetite suppression. It is an endocannabinoid which acts as a neuromodulator centrally and peripherally. An over-expression of endocannabinoid receptors is seen in the obese which is believed to enhance stimuli for food intake. Rimonabant interferes with these signals resulting in appetite suppression and weight loss[461]. It was endorsed for restricted use in obese individuals by the EMEA in June 2006, following the results of four randomised controlled trials which studied over 6000 subjects and looked at various aspects of cardiovascular disease. The "Rimonabant In Obesity" (RIO) trials focused on overweight and obese individuals (RIO Europe and RIO North America), individuals with type 2 diabetes (RIO-Diabetes) and those with lipid disorders (RIO Lipids). In all four trials the use of rimonabant in conjunction with dietary measures demonstrated improvements in weight, abdominal adiposity, lipid profile, blood pressure, and other cardiometabolic factors. 20mg of rimonabant daily resulted in an average weight loss of 8kg in a year as compared to 3kg in the control group. The diabetes group had a weight loss of 5.3kg (5.4% of baseline body weight) as compared to 1.4kg in the placebo group[419;473;550;551].

Within the RIO trials, a 7% withdrawal rate on the 20mg dose due to psychiatric problems, mainly depression, was noted. As individuals with known significant depression had been excluded from the trial, this figure was considered excessive and the FDA investigated further. A meta-analysis looked at data from over 4,000 individuals and concluded that patients given rimonabant were 2·5 times more likely to discontinue treatment because of depressive mood disorders than those on placebo, and that another significant proportion experienced symptoms of anxiety[97]. In October 23, 2008, the EMEA released a statement that its Committee for Medical Products for Human Use (CHMP) had concluded advising that rimonabant be suspended from the UK market as its benefits no longer outweighed its risks[22].

1.10.4. Glucagon Like Peptide 1 Agonists / Analogues

A new class of agents for the management of individuals with type 2 diabetes are the glucagon like peptide 1 (GLP-1) mimetics, Exenatide, Liraglutide, and more recently Bydureon. GLP-1 is a hormone secreted from gut enteroendocrine cells known as an incretin. Incretins were noted in the 1970s to have insulinotropic properties and to regulate insulin levels[546]. Incretins are believed to be responsible for up to 70% of post-prandial insulin secretion[240].The GLP-1 receptor when activated engages in a series of events via adenylate cyclase and exerts physiological actions within pancreatic islets, liver, adipose tissue and skeletal muscle, which have been identified to

- stimulate insulin secretion
- increase beta cell proliferation
- enhance intestinal somatostatin production
- inhibit glucagon secretion
- promote hepatic glucose uptake and glycogenesis
- inhibits acid secretion and delays gastric emptying[127;128]

These actions appear to be glucose-mediated making incretins attractive as they are also less likely to promote insulin over-secretion and hypoglycaemia[196]. Human GLP-1 is rapidly degraded by dipeptidyl peptidase IV making it ineffective unless given as a constant infusion, and thus the search for a longer acting alternative.

Exenatide, the first GLP-1 mimetic was released on to the market in 2005. It is derived from exendin-4, a naturally occurring GLP-1 receptor agonist originally found in the saliva of the Gila monster. It is 53% homologous with human GLP-1 but lacks the site recognised by dipeptidyl peptidase IV, allowing it to attach to GLP receptors, but avoid rapid degradation thus increasing the half-life. It is administered as a twice daily subcutaneous injection. Liraglutide, marketed in 2009, is an analogue of human GLP-1 with an amino acid substitution and the addition of a C-16 palmitic acid side chain which makes it resistant to degradation. It is administered as a once daily sub-cutaneous injection[81;545]. Other companies have released or are in the process of releasing their version of a GLP-1 mimetic, including bydureon, a one weekly extended-release exenatide and once daily lixisenatide.

GLP-1 agonists improve HbA1c, post–prandial glucose levels, and insulin requirements. Cardiovascular outcome trials are currently ongoing[82;117;148;383]. In randomised controlled trials these agents have resulted in 2 to 4kg weight loss in excess to placebo, sulphonylureas, metformin or thiazolidinediones[262;439]. They have also been demonstrated to improve blood pressure, as well as total and LDL-cholesterol[533]. The exact mechanism for weight loss is to be clearly identified but is currently believed to be a combination of delayed gastric emptying, premature satiety and a central anorectic effect from stimulation of the brain GLP-1 receptors.

GLP-1 mimetics are currently licensed for use in type 2 diabetes, but a trial is currently on-going investigating the potential for use in obese, insulin resistant individuals. SCALE (Satiety and Clinical Adiposity – Liraglutide Evidence in Non-Diabetic and Diabetic Subjects) is currently on-going having randomised individuals to placebo or liraglutide 0.6, 1.2 or 3mg[394]. The results may help provide further evidence to justify using these agents in obese insulin-resistant patients without diabetes[306;533].

GLP-1 agonists' side effects are mainly gastrointestinal, but hypoglycaemia can occur when administered with insulin secretagogues. Liraglutide and Exenatide extended-release in animal studies were noted to enhance thyroid C-cell hyperplasia, raise calcitonin levels, and potentially promote medullary thyroid cancers. Consequently the FDA, placed a black box warning on both drugs, encouraging both physicians and patients to assess pros and cons prior to use[62].

1.10.5. Metformin

Metformin is a biguanide which is botanically linked to *Galega qfficina (*Goat's rue*)* a herb which had in medieval times been used for the treatment of diabetes. Derivatives of biguanides were initially introduced in the 1920s but did not gain popularity due to side-effects and the discovery of insulin which was much more effective in controlling blood sugars. Reintroduced in the 1950s, metformin has since become one of the commonest used oral hypoglycaemic agents, and is first line therapy for individuals with type 2 diabetes in the UK[21;45;46].

The exact mechanism of action of metformin remains unclear. It is believed to reduce insulin resistance by enhancing glucose uptake by both peripheral muscles and liver and reducing gluconeogenesis. Metformin is effective at reducing blood glucose levels, without causing hypoglycaemia or weight gain making it a desirable agent compared to the sulphonylureas. Its side-effects are mainly gastro-intestinal. The incidence of lactic acidosis, which was the reason for the withdrawal of other biguanides, is low in metformin[7;86].

Metformin was the first hypoglycaemic agent to suggest a positive effect on cardiovascular outcomes[25]. Not only is it effective in improving glucose control, it appears to have an independent positive effect on total and LDL-cholesterol, It is commonly used in polycystic ovary syndrome where it appears to improve insulin resistance and help regulate menstrual cycles and ovulation.

In addition to its above benefits, metformin is now being increasingly used to assist with weight loss. Studies in overweight or obese individuals with type 2 diabetes have demonstrated that metformin resulted in appetite suppression and weight loss, an effect that was clear when compared to sulphonylureas or insulin[85;315;344;594]. In obese hyperinsulinaemic teenagers on hypocaloric diets, metformin achieved a greater reduction in weight (6.5% vs. 3.8%, $p<0.01$) and body fat, as well as improved insulin sensitivity[276]. A systematic review of studies looking at weight loss with metformin in overweight or obese individuals without type 2 diabetes reported that although metformin might enhance weight loss in individuals with a BMI >35kg/m^2, they were unable to conclusively confirm this [321].

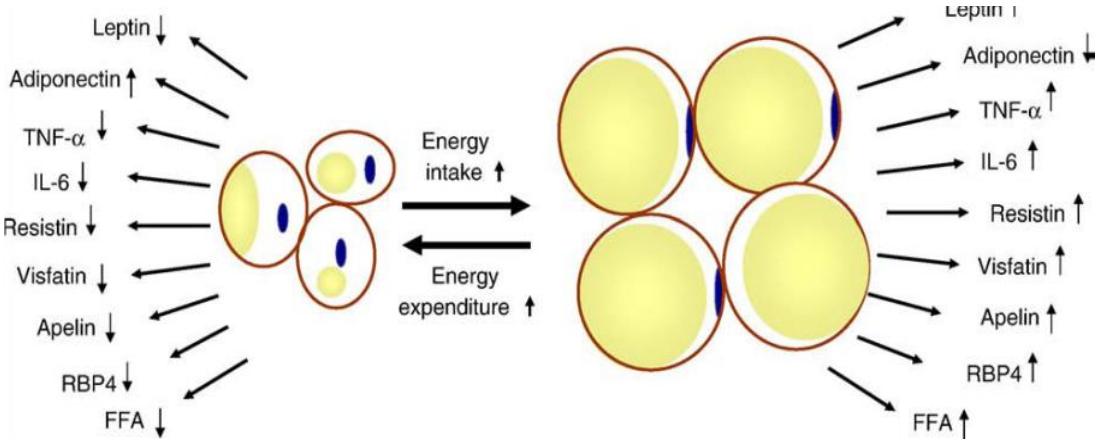
Certainly, over the years, it has become a popular agent used in increasingly large numbers of individuals who are overweight and have some degree of insulin resistance.

2. CHAPTER 2: Adipocytokines

2.1. Introduction & Definition

The precise mechanisms linking obesity, insulin resistance, dyslipidaemia and hypertension to the development of the metabolic syndrome are ill-defined, but it is noted that many of these factors are associated with higher than normal circulating levels of inflammatory markers, "cytokines" which are associated with atherogenesis[63;435]. Cytokines are molecular substrates secreted by a cell to alter its own function (autocrine activity) or that of adjacent cells (paracrine activity). They have a role in immunomodulation. Cytokines are elevated in both obesity and metabolic syndrome with the underlying cause likely to be adiposity. Adipose tissue develops in many sites throughout the human body aggregating in areas of loose connective tissue, such as the subcutaneous layer dividing muscle and skin. It may accumulate around internal organs particularly abdominal viscera and the heart. Previously adipose tissue was believed to be solely an inactive storage facility whose function was to provide insulation, mechanical support and storage of surplus energy. Studies have demonstrated it to be a metabolically active organ with its own biologically active molecules that are specific for its various targets[342;408](Figure 13).

Figure 12: An overview of secretion of adipocytokines in adipose tissue under normal and obesity conditions[604].



Adipose tissue is classified into white and brown. White adipose tissue makes up the larger component and is the major site for energy storage. A white adipocyte is composed of a single fat droplet with a peripheral nucleus. It is regulated by a cascade of hormones, including glucagon and catecholamines, which activate or deactivate hormone sensitive lipase which in turn regulates the influx and efflux of free fatty acids and thus energy supplies for gluconeogenesis. White adipose tissue has a capacity for major expansion which is a feature unique to it allowing it to potentially become the largest body organ. Brown adipose tissue is linked to thermogenesis and preservation of body temperature and is thus seen in abundance in situations where heat generation is required such as in animals that hibernate or in neonates including humans.

Table 14: Action of Cytokines

Adipocytokine	Site of production	Actions
Adiponectin	Adipose tissue Skeletal muscle	Inverse relationship with obesity Enhances insulin sensitivity Reduces gluconeogenesis Anti-atherogenic Anti-inflammatory /Anti-thrombotic Beneficial effect on dyslipidaemia
Leptin	Adipose tissue (subcutaneous) Placenta Ovaries Skeletal muscle, Stomach Bone marrow Pituitary Liver	Inhibits appetite Increases metabolism and energy expenditure Enhances insulin sensitivity Inhibits lipogenesis Stimulates pro-inflammatory cytokines Increases platelet aggregation
Resistin	Macrophages Adipose tissue Immune cells	Augments insulin resistance Stimulates smooth muscle cell proliferation Pro-inflammatory
Visfatin	Visceral adipose tissue Bone marrow Liver Muscle Lymphocytes	Enhances insulin sensitivity Insulin-like properties
Retinol Binding Protein 4	Adipose tissue	Augments insulin resistance
Plasminogen Activator Inhibitor 1 (PAI-1)	Adipose tissue Endothelium	Augments insulin resistance Inhibits fibrinolysis Promotes atherosclerosis

Unlike white adipocytes the brown adipocyte is made up of several small fat droplets, has a larger number of mitochondria, a higher capillary supply and a greater oxygen requirement. Cytokines secreted by adipose tissue are collectively known as adipocytokines (Table 14). They were first described in 1994 with the discovery of leptin, a satiety regulator, which demonstrated that adipose tissue was capable of producing signals that enabled it to regulate food intake and energy balance, and thereby affect the body's nutritional status. Other largely adipose-derived factors soon followed: adiponectin, resistin, visfastin and plasminogen activator inhibitor 1 (PAI-1). Adipocytokines interact with several organ systems, their systemic influences during times of dysregulation potentially linking them to the insulin resistance, metabolic dysfunction, and inflammation in MS[249].

In more recent years another category of fat has become recognised. This is known as ectopic fat and has been defined as the collection of fat (triglyceride) deposits in areas that are not commonly recognised to be associated with fat/adipose tissue (i.e. liver, skeletal and cardiac muscle, and pancreas). The exact mechanism behind the formation of ectopic fat is still poorly understood but adipose tissue dysfunction and the saturation of subcutaneous adipose tissue are both believed to be causative factors. A disproportion in the rate of accumulation of free fatty acids and metabolites, and their oxidation leads to a build-up in intracellular lipid which depending on the organ they affect, may negatively impact on its function (i.e. non-alcoholic fatty liver, or pancreatic exocrine dysfunction)[318;503]. A group from Newcastle have recently demonstrated that a strict hypocaloric diet (<600kcal/day), could reduce pancreatic and hepatic triglyceride stores, and reverse the metabolic abnormalities associated with type 2 diabetes[4].

Ectopic fat has been divided into two types depending on its area of activity. There is (1) ectopic fat with systemic effects (intramuscular, fatty liver, visceral adipose tissue) which is believed to have a role in insulin resistance and metabolic dysfunction, through the release of adipocytokines and inflammatory markers; and (2) ectopic fat with local effects (perivascular fat, pericardial fat, myocardial

[4] Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol, E. L. Lim & K. G. Hollingsworth & B. S. Aribisala & M. J. Chen & J. C. Mathers & R. Taylor. Diabetologia. DOI 10.1007/s00125-011-2204-7

steatosis), which directly affects the organ to which it is confined resulting in reduced muscle contractility or vascular hardening.

Many include visceral adipose fat as part of ectopic fat and have thus associated it with an increase in metabolic and cardiovascular risk. Studies have demonstrated that lifestyle changes and improving insulin-sensitivity does have a positive effect on the quantity of ectopic fat. .Much work is still needed to fully appreciate and quantify the effect that the various deposits of ectopic fat contribute to insulin resistance and the inflammatory process[75].

2.2.Adiponectin

Adiponectin is a 244–amino acid protein synthesised and secreted almost exclusively by white adipocytes. It circulates mainly in two forms: hexamers of relatively low molecular weight and larger high molecular weight, multimeric structures. The ratio between the two forms is believed to determine adiponectin's physiological activity[181]. Its secretion is likely to be hormone regulated. IGF-1 stimulates its production whereas insulin, TNF-α, endothelin-1, and glucocorticoids appear to be suppressive[188].

Unlike the other adipose-related cytokines, adiponectin has an inverse relationship with obesity with levels rising during weight loss but falling with weight gain. It appears to have a role in metabolic dysfunction as well as anti-atherogenic and anti-inflammatory effects. Adiponectin has an inverse correlation to hyperinsulinaemia, insulin resistance, obesity, BMI, hypertension, dyslipidaemia, and hyperglycaemia. Its correlation to insulin resistance appears to be stronger than for obesity or degree of body fat. Low plasma adiponectin was an independent risk factor for development of type 2 diabetes (but not for obesity) [31;342;484;591]. Although negatively associated with general adiposity, adiponectin levels differ according to the distribution of fat with levels being lower in visceral adiposity or high waist to hip ratios[249;302]. Females have higher adiponectin levels than males which may be a reflection on their fat distribution which tends to be more peripheral and subcutaneous. Such associations suggest that adiponectin levels may be affected by sex hormones in addition to the pattern of fat distribution although the evidence is variable[236;508].

Studies in mice have demonstrated an improvement in insulin resistance when adiponectin was administered to obese rodents or ones with type 2 diabetes. Other studies combining both adiponectin and leptin demonstrated that while both agents improved insulin sensitivity, the combination had additive effects with complete resolution of insulin resistance in some[293;585].

Plasma concentrations of adiponectin are lower in people with CVD than in controls, even after matching for BMI and age. Individuals with hypoadiponectinaemia (<4μg/ml) are likely to have multiple metabolic risk factors and an increased risk of CVD[300]. At the same time those with higher adiponectin levels have reduced long term risk of myocardial infarction. These differences in risk persist after adjustment for family history, BMI, alcohol intake, history of diabetes and hypertension, haemoglobin A1c, hsCRP, and lipoprotein levels[423]. In obese individuals adiponectin levels decreased incrementally with each additional component of MS.

The mechanisms by which adiponectin may influence insulin resistance is not fully understood. It is believed to be linked to a reduction in circulating fatty acids and triglyceride load through adiponectin's ability to stimulate intra-muscular fatty acid oxidation[585] or to activate AMP-activated protein kinase, increasing intramuscular and adipose tissue glucose uptake. In addition to its effects on obesity, insulin resistance and associated markers of CVD, adiponectin plays a potential anti-thrombotic and anti-inflammatory role. In human studies it is negatively correlated to hsCRP levels, which are associated with inflammation and atherogenesis[355;403]. In vitro adiponectin inhibits pro-inflammatory TNF-α and interleukin-8 induced synthesis of adhesion molecules which trigger a series of events which suppress the production of pro-inflammatory cytokines and enhance the secretion of the anti-inflammatory cytokine interleukin-10[299;402;520].

Controversially, a few studies have implied that high adiponectin levels are not protective for secondary CVD protection and may actually be associated with an increased mortality. These might suggest that in time of disease, a counter-regulatory increase in adiponectin occurs, representing a defence mechanism against inflammatory conditions[119;282;309]. Further studies are required to elucidate adiponectin's role in disease.

2.3. Leptin

Leptin, was the first adipocytokine to be discovered in 1994. It is produced by the leptin gene, whose name is derived from the Greek word *"leptos,"* meaning "thin." Leptin is an 167-amino acid protein, synthesised and secreted mainly from white adipose tissue as an afferent signal molecule that interacts with the appetite and satiety centres in the brain to regulate body weight, inhibit food intake and increase energy expenditure, enhance thermogenesis and metabolic rate[12;425;490]. Circulating leptin levels correlate with body fat stores although the mechanisms regulating its rise when overfeeding and fall in starvation appear to be unrelated to adiposity. Leptin receptors are located in the central nervous system, adipocytes and endothelial cells where they facilitate pathways which are essential for regulating appetite, modulating hepatic gluconeogenesis and gonadotrophin secretion[438;600].

Leptin deficiency or receptor dysfunction causes hyperphagia, obesity, hyperinsulinaemia and insulin resistance in both animal and human studies. Hyperleptinaemia can also be present in obesity. While inherited leptin deficiency or mutations occur in the morbidly obese, insulin resistant (without diabetes), and sexually dysmorphic, these are rare accounting for only a tiny percentage of obese individuals, and respond positively to recombinant leptin therapy[12;147;434;438;490]. Most obese individuals are considered leptin resistant, with bodies adapting to high circulating levels and failing to trigger the satiety effect.

In addition to feeding, factors affecting leptin levels are exercise, raised metabolic rate, which hypothetically should induce hypoleptinaemia (although results remain controversial), and exposure to the cold, a possible self-preservatory effect during hibernation[219;289;411;415;600]. In men, hyperleptinaemia may be a predictor of type 2 diabetes, but female studies have failed to show a similar association.

Leptin inhibits insulin biosynthesis and secretion in pancreatic β-cells. Insulin stimulates leptin secretion from adipose tissue. The two are interlinked in a feedback mechanism that precludes leptin resistance when dysregulated and contributes to hyperinsulinaemia and type 2 diabetes in overweight individuals[480]. Leptin may

contribute to worsening cardiovascular risk by counteracting the antioxidative actions of insulin on free fatty acids. Studies have reported hyperleptinaemia to be a predictor for future CV events in those with a previous event or family history[287;363;507]. Controversially some studies have implied that leptin may have cardioprotective properties and indeed hypoleptinaemia has been linked to increased cardiovascular mortality[420]. Consequently some have wondered if leptin's contradicting properties exist within a balance with anti-atherogenic and insulin-sensitising properties on one side and atherogenic and insulin resistance promotion on the other. Obesity and MS clearly tip the scales towards oxidative stress and a pro-inflammatory status promoting cardiovascular disease and type 2 diabetes.

Leptin is also involved in many physiological processes. It modulates T- cell immune response, stimulates proliferation of T-helper cells, tumour necrosis factor, and production of pro-inflammatory cytokines. Leptin has also been found to up-regulate the production of endothelin 1 (ET-1), a potent vasoconstrictor and mitogen, in human umbilical vein endothelial cells and this may explain the relationship between adipose tissue and obesity-associated hypertension and atherosclerosis[434]. Its role in atherosclerosis is still not fully determined. Hyperleptinaemia has been identified to be associated with increased platelet aggregation[381]. In *in vitro* or animal studies, different atherogenic properties, including increased oxidative stress, impairment of vaso-relaxation, and increased thrombosis, have been described[505;506;562].

The role of leptin for the future management of obesity and cardiovascular risk is still being considered. Treatment with recombinant human leptin reverses hyperphagia, obesity, hypogonadism, and T-cell immunodeficiency in those with congenital leptin deficiency. In addition, leptin replacement for the management of lipodystrophy is a promising therapeutic approach. Disappointingly, most obese individuals, for unknown reasons, are resistant to the satiety and weight-reducing effect of leptin. Studies are on-going looking at how to manipulate the neurohormonal pathways to make it an effective anti-obesity pharmacotherapy[145;146;238].

2.4. Resistin

Resistin (for resistance to insulin) was first described in 2001, whilst researching rosiglitazone[514]. It is a 114-amino acid polypeptide hormone belonging to a family of cysteine-rich proteins. Human resistin is only 59% homologous to the mouse molecule, which may account for variability between animal and human studies, limiting the full understanding of its mechanism of action. Although secreted mainly from white adipose tissue in mice, resistin, in humans, is primarily secreted from macrophages, then bone marrow, lungs and at a low level, adipocytes[249;592]. Resistin is implicated as a link between obesity and diabetes through the augmentation of insulin resistance. In mice, resistin antagonizes insulin-mediated glucose uptake within peripheral cells and enhances hepatic gluconeogenesis. Treatment with recombinant resistin resulted in insulin resistance, whereas administration of an anti-resistin antibody enhanced insulin sensitivity in obese and insulin-resistant animals[514]. It is supposedly regulated by nutritional status with levels rising in response to feeding and acute hyperglycaemia and falling in fasting and hyperinsulinaemia [226;365;514;515].

Some animal studies have demonstrated that resistin levels are elevated in *ob/ob*, *db/db* and diet-induced obesity model rodents while others failed to demonstrate similar findings. In some human studies, resistin mRNA levels in adipocytes were increased in morbidly obese subjects when compared to lean controls with levels correlating positively with BMI and visceral fat area but not waist-hip ratio[109;118;472]. In contrast, other studies have shown no correlation between resistin levels and body fat, visceral adiposity or BMI[230;496]. In children serum resistin levels were similar in the obese and nonobese and did not change with body mass index over time[187;443].

As resistin is expressed in only small amounts in human adipose tissue, its role in obesity and insulin resistance remains unclear. Some have reported that resistin is secreted mainly from visceral adipose tissue, particularly “omentum” and highly correlates to insulin resistance, arguing that this relationship links it to obesity[364;365]. In addition weight loss studies, have reported reductions in resistin with

moderate weight loss and in those treated with gastric banding[548;555]. At the same time others have reported that resistin is not preferentially secreted from visceral tissue but is equally present in gluteo-femoral fat and failed to show any correlations to markers of obesity[301;316;496]. Resistin has been positively correlated to insulin resistance with levels reported to be 20% higher in those with type 2 diabetes by some human studies while others have failed to show any association with markers of insulin resistance adding to the confusion of resistin's role[171;257;316;590]. Even the effect of insulin sensitizers on resistin was variable with rosiglitazone and troglitazone both showing signs of down regulation, and metformin and pioglitazone enhancing resistin expression[172;175;301;374].

As an inflammatory agent, resistin activates vascular endothelial cells and stimulates smooth muscle cell proliferation resulting in atherosclerosis[247]. Studies have confirmed resistin levels to be increased in inflammatory conditions such as rheumatoid arthritis and atherosclerosis, and reported that inflammatory markers such as TNF-α, CRP and interleukin-6, were regulated by resistin implying that hyperresistinaemia may be a mediator of inflammation rather than a by product[442]. Cardiovascular studies have identified it as a risk factor for females with coronary heart disease and a predictor of future myocardial infarction but not ischaemic stroke[422;566]. Interestingly the studies have shown a positive correlation among females but not in males suggesting a possible gender effect[112;392].

2.5. Visfatin

Initially believed to be preferentially expressed in visceral fat, visfatin was first described by Fukuhara in 2005 who considered it to have insulin-mimetic functions[173;442;442]. After sequence analysis, visfatin was found to correspond to the pre-B cell colony-enhancing factor 1 (*PBEF1*) previously described by Samal[468], which was responsible for lymphocyte maturation and inflammatory regulation. Since, it has been identified as nicotinamide phosphoribosyl transferase, an enzyme responsible for nicotinamide adenine dinucleotide (NAD) biosynthesis[186]. In addition to adipocytes, visfatin is expressed in skeletal muscle, liver, bone marrow and lymphocytes where it has been linked to inflammatory responses, inhibition of apoptosis, and maturation of vascular smooth muscle cells[599]. Visfatin levels are elevated in individuals with type 2 diabetes, glucose intolerance, gestational diabetes and severe obesity (BMI >40). It is also noted to be elevated in sepsis, established coronary artery disease, and unstable angina with levels appearing to be higher among those with established evidence of atherosclerosis or MS[334;601].

Visfatin's role in insulin regulation and insulin resistance is controversial. It was first reported to exert insulin-like activity in a number of cells, and to upregulate insulin secretion by 46%. In mice, an injection of visfatin lowered blood glucose, while mutations within the visfatin gene can cause glucose intolerance mainly through insulin deficiency and a reduction in glucose uptake intracellularly[33;93;173;248;447]. Further studies failed to show similar findings casting doubt over visfatin's role and ending with Fukuhara retracting their original findings[77;174;447]. While supposedly regulating glucose and insulin secretion visfatin appears to be regulated by the same, with glucose infusions increasing visfatin levels and insulin or somatostatin suppressing it[207].

In patients with type 1 or type 2 diabetes, visfatin levels were significantly elevated in those with a longer duration of disease. In those with type 2 diabetes, there even appeared to be a positive correlation with glycated haemoglobin levels (HbA_1C)[336], although other studies seemed to indicate the inverse particularly in type 1 diabetes[536].

In some human studies, visfatin expression within visceral adipose tissue appeared to positively correlate with BMI, while subcutaneous fat visfatin negatively correlated with BMI implying a link between visfatin, visceral adiposity and thus insulin resistance. Others studies have failed to demonstrate the same differences reporting that visfatin expression was equal in all fat tissue, while some failed to establish any relation to other anthropometric measures or insulin resistance[61;92;407;599]. Visfatin plasma levels increase in response to a high-fat intake, indicating that it may play a significant role in diet- or obesity-induced insulin resistance[206;210;214;554]. Responses to weight loss studies have been variable with some reporting visfatin levels falling and others rising in response to weight loss through dieting or bariatric surgery[66;93;182;210].

As a pre-B cell colony-enhancing factor 1, visfatin is believed to be an inflammatory mediator. It has been shown to positively correlate to interleukin-6, CRP and TNF-α, and has been reported to be elevated in inflammatory conditions (i.e. rheumatoid arthritis, acute lung injury, inflammatory bowel disease and myocardial stress). In rodent studies it was implied that an acute infusion of visfatin at the time of myocardial injury may be protective but this is yet to be described in human studies[303;327;399].

There are a number of inconsistencies among the different studies of visfatin, and the role of this adipokine in obesity, insulin resistance and inflammation is yet to be clearly defined.

2.6. Retinol Binding Protein 4 (RBP4)

Retinol binding protein 4 (RBP4) is a 21 kDa protein which acts as a specific carrier for retinol in blood. It is one of the proteins that solubilize and stabilize the hydrophobic and labile metabolites of retinoids in aqueous spaces within extra- and intra-cellular spaces. Its physiological function appears to be binding to retinol and preventing its loss via kidneys. It was first reported as an adipocytokine in 2005 during investigations of muscle-related insulin resistance in mice devoid of the glucose-transporting protein (GLUT) 4. Although mainly produced in liver, it is also known to be secreted by adipocytes with enhanced production in obesity, causing speculation of another link for impaired insulin sensitivity[589]. In rodent studies, RBP-4 levels were inversely proportional to the amount of GLUT4 expressed in mice adipose tissue. Insulin sensitivity is improved and GLUT4 restored when circulating RBP4 levels fall, by natural measures or through the administration of a synthetic retinoid, and insulin resistance is induced when recombinant RBP4 administered[195;522].

Cho et al. reported that plasma concentrations of RBP4 were higher in individuals with impaired glucose tolerance or type 2 diabetes than in normal glucose tolerance. No difference in RBP4 levels were noted between those with glucose intolerance and severe diabetes[94]. A different study demonstrated RBP4 to correlate with severity of insulin resistance in obese subjects with impaired glucose tolerance or type 2 diabetes, as well as in non-obese, non-diabetic persons with a strong family history of type 2 diabetes[195]. RBP4 levels rise with increasing numbers of MS components and correlated strongly to BMI, blood pressure, serum triglyceride and HDL-cholesterol levels[195]. RBP4 was reported to be more highly correlated with waist-to-hip ratio and visceral fat than with BMI. However, Janke et al. reported that, RBP4 mRNA is down-regulated in the abdominal subcutaneous adipose tissue of obese women, although circulating RBP4 levels were similar in lean, overweight, and obese females. Exercise may reduce RBP4 levels but only in the presence of improved insulin resistance.

Despite several studies identifying close associations between RBP4 and insulin resistance, a number of others were unable to demonstrate similar findings. In healthy insulin resistant individuals plasma RBP-4 levels failed to correlate with insulin levels or measures of insulin resistance. Another group failed to show changes in circulating RBP4 levels despite a 5% body weight loss although there was positive correlation to GLUT4 expression with down regulation in those losing weight. In a Japanese study Takashima et al could find no correlations of RBP4 levels to fasting blood glucose, waist-to-hip ratio, BMI, systolic blood pressure, or fasting insulin, in their study group, regardless of a family history of diabetes[521]. It remains uncertain as to why the differences exist among similarly conducted human studies.

Of interest is a German study investigating 220 individuals with and without type 2 diabetes, RBP4 levels appeared to correlate with the degree of renal function rather than diabetes, implying that renal function not insulin resistance might affect RBP4 levels. Similar results were reproduced by a different group who reported RBP4 to be affected by renal but not hepatic dysfunction[168;234]. Another study correlated high circulating RBP4 levels with elevated intrahepatic fat deposits and presumed the changes were related to hepatic insulin resistance[511]. Certainly, future studies are needed to clarify how RBP4 affects insulin resistance in rodents, whether it does in man, and if so to define the role it may play in the development of MS, type 2 diabetes, and even CVD.

2.7. Plasminogen Activating Inhibitor type 1 (PAI-1)

Plasminogen activating inhibitor type 1 (PAI-1), a linear glycoprotein with a molecular weight of 48,000, is an inhibitor of plasminogen activators and inhibits fibrinolytic activity[288;490]. Initially believed that PAI-1 was solely synthesised by endothelial cells and the liver, it is now known to be produced in smooth muscle cells, fibroblasts, monocytes/macrophages, endometrium, peritoneum, liver cells, mesothelial cells, cardiac myocytes and adipose tissue (especially intra-abdominal visceral fat which is a major source)[357;465;490]. Its production within adipose tissue is mainly in the stromal compartment and is modulated by macrophages responding to oxidative stress, TNFα, loss of circadian rhythm and metabolic markers such as hyperinsulinaemia, hyperglycaemia, and hypertriglyceridaemia. PAI-1 is mainly stored within platelets from which the active form is released when platelets are stimulated by thrombin. Active PAI-1 is unstable, with a half-life of 30 minutes. For transportation it is usually stabilized by binding to vitronectin.

Elevated plasma PAI-1 levels are associated with the destabilization of coronary disease and development of acute coronary syndromes and myocardial infarction. Raised plasma PAI-1 concentrations are positively associated with myocardial infarction in individuals with stable angina and angiographic evidence of progressive CAD in young men with a history of myocardial infarction. Despite raised plasma PAI-1 levels being identified as a predictor of myocardial infarction, its predictive ability resolves once adjustment for components of MS are considered, implying that MS may be a prerequisite for elevated PAI-1 in individuals prone to atherothrombosis[56].

PAI-1 levels are reported to be lower during the day than at night, an observation which may explain the higher incidence of myocardial infarction in the early morning hours, as PAI-1 activity favours fibrin production by disturbing equilibrium between fibrin generation and fibrinolysis [19]. In addition, PAI-1 has an effect on many other systems including ovarian function, cancer progression and insulin resistance[298;531].

In rodent models, PAI-1 prevented fat accumulation in PAI-1 deficient mice through dietary modification or genetic manipulation and PAI-1 inhibition appeared to improve insulin sensitivity by enhancing intracellular glucose uptake[340]. The lack of increased adiposity was reported to be due to an increase in metabolic rate, total energy expenditure and thermogenesis implicating that PAI-1 may be involved in controlling fat accumulation through a central effect on the hypothalamic paraventricular nucleus which controls satiety[523].

The plasma concentration of PAI-1 is elevated in obese subjects with insulin resistance, type 2 diabetes, or MS, with levels increasing with each increase in components of MS or presence of microalbuminuria[375]. PAI-1 levels also fell with improvement in insulin resistance, reduction in insulin and weight loss[28]. Population studies have shown that raised PAI-1 levels are a predictor for the development of diabetes[154]. In humans, a direct correlation between visceral fat and PAI-1 levels exists, independent of insulin sensitivity, insulin or triglyceride levels[490]. Exogenous insulin was noted to reduce plasma PAI-1 activity in type 2 diabetes without improvement in glycaemic control and suppresses the secretion of both insulin and insulin precursor molecules, such as proinsulin and split proinsulin which have also been noted to affect PAI-1 expression[254;288]. Genetic polymorphisms in PAI-1 have been linked with obesity, insulin resistance, and increased triglycerides in some studies but not in others[377].

Inhibition of PAI-1 is associated with enhanced cardiac recovery following myocardial infarction and reduced aortic wall thickening as promoted by angiotensin II and a high-salt diet in mice. Paradoxically, PAI-1 inhibition was also associated with increased growth of atherosclerotic plaques in mice predisposed to atherosclerosis. In some studies, PAI-1 increased the risk of recurrent myocardial infarction, atrial fibrillation, and progression of coronary atherosclerosis following myocardial infarction while in other studies no association to subsequent coronary events was noted[215]. Some believe that the enhanced expression and release of PAI-1 from visceral adipose tissue is the link between this adipocytokine and cardiovascular disease especially as it is highly associated with adverse metabolic risk factors[16].

PAI-1, the main inhibitor of the fibrinolytic system, could be a component of the MS. The mechanisms linking the two are complex and probably interrelated, with several inducers possibly acting at several sites of synthesis. Studies have indicated that PAI-1 might be involved in the development of obesity, insulin resistance and type 2 diabetes. Further studies are required to better understand this complex interplay.

2.8. C-Reactive Protein

C-reactive protein (CRP) is an acute-phase reactant that is produced in response to acute injury, infection or other inflammatory stimuli. CRP has been shown in numerous cohort and case–control studies to be a measure of underlying systemic inflammation and a strong associate of future cardiovascular events particularly myocardial infarction, ischaemic stroke and peripheral vascular disease, stimulating interest in a possible role for CRP measurement in CVD risk assessment in clinical practice[448;449;451].

Acute inflammation triggers the release of cytokines which in turn stimulate the substantial production of CRP largely through interleukin-6. Once the inflammatory stimulus has passed, CRP levels return to baseline. Many conditions cause mild elevation in CRP such as low grade inflammation, smoking, pollution, hormone replacement therapy and atherosclerosis which are not picked up by standard CRP assays[430]. Newer assays are up to one hundred times more sensitive and capable of measuring these smaller changes. To distinguish it from standard CRP these assays are known as high-sensitivity CRP (hsCRP). CRP has been demonstrated to be a good predictor for CVD risk, and the development of type 2 diabetes[430]. In the Physicians' Health Study men in the highest CRP quartile had an increased risk of MI (3x), and ischaemic stroke (2x), as those in the lowest quartile[450]. Similar results were seen in the Reykjavik Study where over 6000 subjects were studied[106]. The Atherosclerosis Risk in Communities (ARIC) study demonstrated that the risk of CHD incrementally increased depending on the baseline hsCRP with it being 2.5 times higher in those with a baseline level of >3mg/L as compared to <1mg/L. Risk changes were independent of age, gender, and ethnicity[48].

In MS, many studies have confirmed that CRP levels were strongly associated with insulin resistance, blood pressure, plasma glucose, low HDL-cholesterol, and triglycerides. A linear relationship was established between the increasing number of components of MS and rising CRP levels[153;160;596]. The strongest associations were observed between CRP levels, central adiposity, and insulin resistance. The link between MS and CRP was so strong that at one point there had been considerations

to add it as a component of MS. Controversy remains on where best to incorporate it as some studies have failed to show that its addition to current criteria (IDF) would be of benefit. In fact in a Finnish study the addition of hsCRP was potentially an inferior identifier in males[239].

CRP is believed to play a role in facilitating the activation of endothelial cells and promoting progression of atherosclerosis by impairing endothelial vasoreactivity and reducing endothelial nitric oxide synthase activity[155;156;524;524]. In rodent studies, the injection of CRP maintained sustained hepatic CRP expression and increased systolic and mean arterial blood pressures. Previous experiments had confirmed that CRP reduced prostacyclin levels, a known vasodilator, leading to observations that CRP may be a cause of hypertension[202;557]. CRP has been shown to increase circulating levels of PAI-1 contributing to increased thrombin formation. In a study of 1001 males with angiographically proven atherosclerosis, plasma CRP levels were negatively correlated to adiponectin levels confirming results seen in wild-type mice[403].

CRP exerts a proinflammatory effect on monocyte–macrophages activating proinflammatory cytokines, enhancing oxidized LDL uptake as well as inhibiting cholesterol efflux, and activating transcription factors which are pivotal in the activation of proinflammatory genes.

Certainly the evidence supporting CRP as an important marker for enhanced cardiovascular risk among individuals with obesity, MS, or type 2 diabetes is overwhelming but, would targeting CRP help protect against CVD? This is yet to be formally investigated.

3. CHAPTER 3: Dietary Interventions

3.1. Introduction – NCEP recommendations

As the prevalence of obesity increased over the past 20 years, the difficulties faced by overweight patients and their health care practitioners have become apparent. Less than 25% of those attempting to lose weight actually reduce their caloric intake or increase exercise as currently recommended. Those who successfully lose weight have difficulty maintaining the weight loss and will usually re-accumulate the shed weight within two years. Consequently, an industry has opened providing all sorts of weight loss products and services complicating and confusing the choices. Most diets are poorly researched with scanty evidence for their effectiveness. Those which are well researched are hindered by compliance and concordance issues thus limiting an individual's ability to make an informed and safe choice[568]. Diets are an integral part of life with some of the first appearing as part of religious instructions such as the Jewish Kashrut by the prophet Moses, recommending, 'bread, wine, milk, honey; quadrupeds with cloven hooves that ruminate, non-preying birds, and fish with scales, and prophet Mohammed (peace be upon him) in the description of halal food which excludes pork and its by-products, alcohol and non-slaughtered meat[225;384].

The first commercially produced diet was by William Banting (1797-1878) the undertaker who made the Duke of Wellington's coffin. Banting was known to be so obese as to be almost spherical. He had tried numerous diets and treatments including exercise, starvation and purging in order to off-load his terrible weight with no success. Finally, believing he was becoming deaf, Banting presented to William Harvey, an ENT surgeon, who diagnosed his problem to be excessive fat compressing his airways and prescribed a low 'farinaceous' diet (Table 15). Within a year he had lost 50 pounds and 12.5 inches of his waist. The diet Banting was instructed to follow formed the basis of his book, "Letter on Corpulence". which he published for the general public, and is the forerunner to the Atkins diet[225]. In his own words he

mentioned "I can now confidently say that QUANTITY of diet may be safely left to the natural appetite; and that it is the QUALITY only which is essential to abate and cure corpulence".

Table 15: William Banting's diet

William Banting's Diet (1864) (Losing 46lb)	
Breakfast:	Four or five ounces of beef, mutton, kidneys, broiled fish, bacon or cold meat of any kind **except pork.** One small biscuit or one ounce of dry toast. A large cup of tea without milk or Sugar.
Lunch:	Five or six ounces of any fish **except salmon, any meat except pork,** any vegetable except potato. Any kind of poultry or game. One ounce of dry toast. Fruit. Two or three glasses of good claret, sherry or Madeira. (Champagne, port and beer were forbidden.)
Tea:	Two or three ounces of fruit. A rusk or two. A cup of tea without milk or sugar.
Supper:	Three or four ounces of meat or fish as for lunch. A glass of claret, or two. Night-cap (if required): A tumbler of grog (gin, whisky or brandy with water but without sugar) or a glass or two of claret or sherry.

Adapted from "Eat Fat and Grow Slim"[341].

His book became a best seller with the "Banting" being the most popular method for weight loss. Unfortunately his diet was never fully understood by the medical profession and not until the twentieth century was further interest cast in such a direction[50].

Obesity can develop when an imbalance exists between energy intake and energy expenditure, a simple equation that appears to be poorly understood or interpreted. Total energy expenditure can be divided into the following components:

1. Resting metabolic rate, the largest single component of energy expenditure. Approximately 60% to 80% of the variation in resting metabolic rate can be explained by fat-free body mass;
2. The thermic effect of food, or the increase in energy expenditure that occurs after eating; and
3. The energy expended in physical activity which varies due to differences in body mass. The energy expended in physical activity is an important component in understanding both.

Over the centuries, obesity has always been considered to be due to overeating and individual behaviour. Management plans focused on behavioural changes and limiting caloric intake with limited or no success. Individuals continued to increase in weight and researchers focused on looking at modifiable external factors as well as quantity and quality of caloric intake. Epidemiological studies investigating the cause of increasing obesity have identified a number of factors attributed to urbanisation, including industrialisation which promoted the use of automobiles for transport and reduced physical activity, increasing dependency on prepared food, with the availability of reasonably priced, fast, energy-dense food[83]. Where obesity was previously seen in higher socioeconomic groups, more recent trends identify the majority of obese or overweight individuals to be among the lower socioeconomic classes in developed countries, with developing countries still following old trends with the higher socioeconomic groups sporting larger BMIs[504]. These trends appear to be more prominent among the female population, although the difference is less marked in more recent years[360].

Table 16: American Heart Association 2006 Diet and Lifestyle goals

- Balance calorie intake and physical activity to achieve or maintain a healthy body weight.
- Consume a diet rich in vegetables and fruits.
- Choose whole-grain, high-fibre foods.
- Consume fish, especially oily fish, at least twice a week.
- Limit intake of saturated fat to <7% of energy, trans fat to <1% of energy, and cholesterol to <300 mg per day by
    - choosing lean meats and vegetable alternatives;
    - selecting fat-free (skim), 1%-fat, and low-fat dairy products; and
    - minimizing intake of partially hydrogenated fats.
- Minimize intake of beverages and foods with added sugars.
- Choose and prepare foods with little or no salt.
- If you consume alcohol, do so in moderation.
- When eating food that is prepared outside of the home, follow the AHA Diet and Lifestyle Recommendations.

American Heart Association 2006 Diet & Lifestyle Goals for Cardiovascular Risk Reduction – adapted from[324].

Consequently strategies to limit the rise in obesity and its associated cardiovascular consequences required revision, targeting causative factors. Although MS is a complicated multifactorial entity, it is clear that dietary and lifestyle changes are central to its management. A number of recommendations have been set based on what is attributed to be a healthy lifestyle, but there is no clear consensus to what "an ideal diet" is. The American Heart Association published non-specific goals aimed at the general population, including children, which encouraged consuming an overall healthy non-atherogenic diet, aiming to achieve the recommended lipid and blood pressure targets, stop smoking and maintain activity (Table 16). Recommendations by the IDF were equally as vague, including energy restriction, increased physical activity, reduction in fat intake, increased fibre intake, and salt restriction.

The ATP III dietary recommendation for therapeutic lifestyle changes stated that an individual's daily caloric intake be divided as below:-

- Carbohydrate intake: 50-60% of total energy
- Total fat intake 25-35% of total energy
  - Saturated fat <7% of total energy
  - Polyunsaturated fat <10% of total energy
  - Monounsaturated fats <20% of total energy
  - Cholesterol <200mg/day not <300mg
- Protein intake 15% of total energy

ATP III recommended that any carbohydrate intake should be complex carbohydrates including whole grains, fruits and vegetables and daily exercise should be enough to consume 200Kcal per day. Such recommendations are reasonable but focus mainly on reducing LDL-cholesterol and possibly adversely affect other parameters, triglycerides and HDL-cholesterol, which are the abnormalities seen in MS. It is thus appropriate to assume that alternative dietary recommendations may be more suitable in MS. Physicians, dieticians and others working within the health industry should look at alternative methods that may be more rewarding. The population, in general, are disenchanted with current dietary recommendations which they find slow and unrewarding. As a result more and more have turned to alternative regimens which promise a quick fix. The evidence behind these diets is small and it

is therefore difficult to assess which is helpful long term. Of the diets which have particularly gained in popularity, are the low carbohydrate, high fat diets such as the Atkins' diet which has been around since the 1970's and the South Beach diet. In the dietary world interest has particularly turned to the Mediterranean life-style which consists of a diet that contains complex carbohydrates, monounsaturated fats and at least the recommended five portions of fruit and vegetables per day.

3.2. The Effect of Diet on Parameters of the Metabolic Syndrome

Weight loss has a profound effect on a number of cardiovascular risk markers and consequently on MS. A 10kg reduction in weight can potentially reduce total morbidity by 20% in addition to reductions in LDL-cholesterol (15%), triglycerides (30%) and a rise in HDL-cholesterol (8%) [479]. A 5% weight reduction theoretically reduces the age-adjusted overall mortality by 12%. Weight loss of 1kg is professed to reduce systolic blood pressure by 1mmHg with reductions sustained for up to 3 years [41;191;387]. In a study of 41 individuals with MS placed on dietary regimens as per NCEP-ATP III guidelines, two thirds (10 out of 15) of those who achieved >10% weight loss no longer fitted the criteria for MS despite maintaining a BMI that was still considered obese compared to only 20% (5 out of 26) in those who lost <10% total body weight[378]. Another study demonstrated that a 6.5kg weight loss through a very low calorie diet for only 4 weeks could reduce systolic blood pressure (11mmHg), fasting glucose (1mmol/l), triglycerides (1.06mmol/l), and total-cholesterol (0.96mmol/l)[41;88]. In glucose intolerant individuals a combination of diet (low-fat, high-carbohydrate) and exercise achieving 5% weight loss reversed progression to type 2 diabetes with a 39% reduction in incidence of diabetes[39;409]. In the Diabetes Prevention Program, every 1kg weight reduction was associated with a 16% relative risk reduction in diabetes incidence [213]. In the Nurses' Health Study lifestyle changes including lack of smoking, regular exercise, healthy eating and moderation in alcohol intake reduced the risk of diabetes, CVD and CHD by 91, 74 and 82% respectively[245]. Weight loss with the aid of medication has similarly reduced the incidence of diabetes. The "xenical in the prevention of diabetes in obese subjects (XENDOS) study" randomized 3,300 obese individuals without diabetes to treatment with orlistat or placebo. After a 4 year follow-up orlistat reduced the incidence of diabetes by 37%. Twenty-one percent of the participants had impaired glucose tolerance and it was among this group that the effect was best noted[535].

3.3. The Effect of Diet on the Lipid Profile in Metabolic Syndrome

The National Obesity Forum has published on its website the improvements expected from a 10% total body weight loss. For lipids the expected changes are:-

- 10% decrease in total cholesterol
- 15% decrease in LDL cholesterol
- 30% decrease in triglycerides
- 8% increase in HDL cholesterol [5]

In the UKPDS 2,906 patients had lipid profiles measured at diagnosis of diabetes and at 3 months following diet therapy. Average weight loss was 4.5kg, with a subsequent 3mmol/l reduction in plasma glucose, and 2% in HbA1c. Changes in total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were -5%, -6.4%, +2% and -23% in men and -2%, -2.3%, +1% and -13% in women. Greatest changes were noted among those with highest initial values for total cholesterol, LDL-cholesterol and triglycerides and lowest values of HDL-cholesterol. Of note, less than 2% of subjects in the UKPDS were on lipid lowering therapy[346;544]. In a systematic analysis of trials investigating lipid outcomes in weight loss, every 10 kg weight reduction lowered total cholesterol by 0.23mmol/l. Similar changes were seen for LDL-cholesterol and triglycerides but no correlation was noted for changes in HDL-cholesterol. Once more, results were more favourable in those achieving the greatest weight loss [427].

3.4. The Effect of Weight Loss on Inflammatory Markers

Weight loss not only improves classical cardiovascular markers, but helps reverse some of the inflammatory changes seen in obesity. Studies have demonstrated that higher concentrations of inflammatory markers (interleukin 6, TNFα) in obese individuals, positively correlated to visceral obesity, and improved with weight reduction programmes[388;487;603]. An Italian study of premenopausal obese sedentary females placed on a year's programme of diet, exercise and counselling demonstrated reductions in TNFα (-31%), interleukin-6 (-47%), P-selectin (-30%), intercellular adhesion molecule 1 (-26%) and vascular cell adhesion molecule (17%) in addition to improvements in all parameters including insulin and glucose with a total body weight loss of 10% [603]. Another study comparing diet-induced weight loss, exercise, or diet with exercise over 18 months in 300 elderly individuals reported reductions in hsCRP, interleukin-6 and TNFα in all three groups although the reductions were not significant with exercise only[388].

3.5. Standard Dietary Recommendations

"A patient can be treated successfully even though he does not know what a calorie is, what a gram represents or the meaning of the words carbohydrate, protein and fat. Indeed, many of my patients cannot read or write. But I think if one has a disease, it is more fun to know something about it." —E.P. Joslin, MD, 1934[270;351]

Recommendations for weight loss and particularly MS have traditionally focused on low fat intake with overall fat intake making up 15-20% and carbohydrates 65% of the total daily caloric intake. Dietary fat is the most energy-dense macronutrient in the diet, providing 9 calories per gram, as opposed to 4 calories per gram for carbohydrate or protein. Fat is considered to add flavour and palatability to food encouraging a greater level of consumption. Additionally, mechanisms behind fat absorption and metabolism are more efficient than those for carbohydrate and protein requiring less caloric expenditure and thus providing more for storage. In a meta-analysis of studies focusing on weight loss and dietary composition Bray and Popkin concluded that high fat diets encouraged obesity by enhancing passive over-consumption of energy and increasing the energy density of the diet[70]. They felt that low fat isocaloric diets were likely to be as effective if not more so than their counter-parts on a high fat diet partly through enhanced satiety as low fat diets tended to contain more complex carbohydrates. Astrup et al, in an analysis of 16 trials, reported similar results, claiming that a reduction in fat intake, without calorie adjustment, did result in greater weight loss than isocaloric higher fat diets[37;38;149].

In the Women's Health Initiative of approximately 49,000 females, half were placed on dietary intervention including a reduction in fat intake from 30 to 20% of total daily calories, increased fruit and vegetable consumption to five or more daily servings, and increasing grains to six or more daily servings, while half had no dietary change. Women in the intervention group lost on average 2.2kg weight in the first year ($p<0.001$) and maintained the weight for the 7.5 years of follow-up[242]. Fat analysis from the self-completed food frequency questionnaires confirmed that a 5% increase in saturated fat increased the risk of CHD by 15%, as compared to the

equivalent carbohydrate intake in calories thus strengthening the argument for low fat diets or substituting unhydrogenated unsaturated fats for saturated fats[246;413].

One disadvantage of carbohydrate-loaded diets is that they contribute to increasing serum triglycerides and reducing HDL-cholesterol levels. Forty-six subjects with MS, randomised to a low-fat, complex-carbohydrate or low fat simple carbohydrate diet for six months, lost more weight on complex carbohydrates with no change in LDL-cholesterol, an increase in triglycerides (higher with simple carbohydrates), and a fall in HDL-cholesterol[429]. Reaven and colleagues studied low-fat, high-carbohydrate diets and reported increases in post-prandial glucose, insulin, triglycerides and VLDL-cholesterol and reductions in HDL-cholesterol. They felt that the national dietary recommendations were potentially harmful to individuals with diabetes or insulin resistance and could enhance cardiovascular risk[104;105;330;333]. A review of carbohydrate-induced dyslipidaemia confirmed that hypertriglyceridaemia did sequentially increase with incremental increase in dietary carbohydrate content. Although reductions in total and LDL-cholesterol levels were demonstrated, there was an increase in the small dense atherogenic LDL subtypes. Regular moderately intense exercise could negate these changes with evidence attained from studying athletes who consumed copious amounts of carbohydrate while training and middle aged men who regularly endurance-trained[232;580;581]. Refined high carbohydrate intake, has been shown to be associated with elevated blood pressure and MS[43;602]. Dietary analysis from the Nurses' Health Study associated a high glycaemic load intake with a greater prevalence of cardiovascular risk factors [333].

Although a reduction in fat intake with enhanced carbohydrate intake may promote weight loss and reductions in LDL-cholesterol, there is risk for substituting refined sugars which even when isocaloric may promote hyperinsulinaemia, dyslipidaemia and a worsened metabolic picture.

3.6. Low Glycaemic Index/Load Diets

3.6.1. Definition of Glycaemic Index and Glycaemic Load

The Glycaemic Index (GI) is expressed as a percentage, but it really represents the relative rate of entry of glucose from the gut into the bloodstream compared with the rate of entry when the glucose is derived from digestion of a reference carbohydrate. The GI is defined as the ratio of:

$$\frac{\textit{Integrated increase in blood glucose in a 2h period after ingestion of known quantity of a test food } \mathbf{x100}}{\textit{Integrated increase in blood glucose level in a 2h period after ingestion of known quantity of a *reference food}^{5}}$$

Carbohydrates are scored as high GI if they score >70, moderate if between 40 and 70, and low if <40. As the glycaemic index is a marker of the speed glucose is absorbed into the body, foods with a low GI e.g. beans, grains and most vegetables, which are absorbed slowly over a prolonged period, are meant to be better for weight management and cardiovascular risk. Foods high in the glycaemic index e.g., white bread, most cereals and sweets are rapidly absorbed and may result in fluctuations in blood glucose levels that could potentially be diabetogenic or inflammatory. The definition was introduced in the 1980s as a means for predicting post-prandial glucose levels and combating glucose intolerance. It built on Burkitt and Trowell's dietary fibre hypothesis which stated that foods that are more slowly absorbed (low GI) may have metabolic benefits in relation to diabetes and to the reduction of CHD risk[79;263;264]. The GI is good for qualifying a type of food, but even low GI foods can promote weight gain and dysmetabolism if eaten in large quantities (i.e. low GI spreads are glucose-deficient but fat rich), and weight reflects calories.

[5] The initial reference food used was glucose, but has now been changed to white bread

Therefore the Glycaemic Load (GL) is a better predictor of the impact of carbohydrate-containing food on post prandial insulin levels as it is a measure of both quality (GI) and quantity of carbohydrate ingested.

Glycaemic load (g) = (Glycaemic index of food (%) X Carbohydrate (g) of that food) / 100%

The increased glycaemic load seen in today's diets is partly due to the increased consumption of refined carbohydrates. Although GI is a tool which alerts health-conscious individuals to alternative beneficial carbohydrates, it has been criticised in causing more confusion and dietary restriction, encouraging failure and defeatism. The use of GI in mixed meals is limited as the differences in glycaemic indices between foods is lost when combined[185]. High GI carbohydrates will dilute the effect of the lower GL components. Fat within a meal can is believed to alter glycaemic response although some studies in which 8–24g fat was fed in mixed meals containing 38–104 g carbohydrate, reported minimal effects on the predicted glycaemic response[264;575].

3.6.2. Benefits of Low Glycaemic Load

The GL of a meal influences appetite through its effect on hormonal regulation. Many studies have confirmed that ingestion of meals containing carbohydrates low in GL reduced the average caloric intake by 20% in comparison to the high GL diets.

In randomised crossover studies subjects placed on low GL diets experienced less hunger, reduced their total caloric intake by 25% and improved their basal energy expenditure. In addition, serum triglyceride levels dropped by 35%, while these increased by 28% in the high GL groups[60;129]. Other studies have proven that low GL diets were more effective at weight loss, improved cardiovascular risk and reduced the hyperinsulinaemia associated with insulin resistance, a key factor in MS. There are knock-on favourable changes in other parameters of MS including blood pressure, dyslipidaemia and markers of inflammation[67;317;333;339;571]. Data from NHANES III

demonstrated an inverse relationship between GL & HDL cholesterol, and GI & HDL-Cholesterol[161]. A cross-sectional analysis of people without diabetes taking part in the Health Worker Cohort Study, described the improvement in lipid profiles by being on low GI or GL diets in 5,000 plus participants[122]. Similar results reported in the Nurses' Health Study where higher GL diets were associated with increased risk of CHD. Interestingly the relationship was more obvious in those with higher BMIs despite adjustments being made for other risk factors[333].

Two systematic Cochrane reviews by Thomas and Elliott assessing the effect of low GI and GL diets on obesity, the overweight and diabetes concluded that obese individuals following low GL diets experienced more weight loss and improvements in lipid profiles, glycaemic control, and total fat mass, than those on low-fat, high-carbohydrate diets, regardless of whether the intake being hypocaloric or *ad libitum*[527;528].

In a randomised controlled trial comparing the effects of a low GL diet to a low fat diet, individuals with glucose intolerance were more responsive to a low GL regimen while no similar advantage was seen in those with normal glucose tolerance, suggesting that low GL intake may be better suited for those with hyperinsulinaemia or glucose intolerance[132]. In a meta-analysis of 14 randomised controlled trials the positive effect of low GI intake for individuals with diabetes was equal to the benefits provided by additional post-prandial hypoglycaemic agents reducing glycated haemoglobin by an additional 0.5% as compared to standard diets[67;132].

In the Zutphen study, the group implied that the benefits of GL are only seen among females when failing to demonstrate and an association between low GI intake and lipid profile, hyperinsulinaemia or glucose levels in men aged 64-84years[549]. The EPICOR study, a cohort of the European Prospective Investigation into Cancer and Nutrition study, reported that Italian women with high GI intake had a higher risk for CHD, but similar findings were not demonstrated for men[494]. Reports from the National Diet and Nutritional Survey suggested that the lack of association may be age related as the group failed to display any benefits of a low GI diet on weight loss or cardiovascular risk reduction among those aged 65 or over[368].

By reducing GI and GL within dietary components, there is a natural tendency to increase protein and fat intake which can be beneficial, depending on the type. Diets rich in protein improve HDL-cholesterol to triglyceride ratios and promote satiety[312]. Monounsaturated fatty acids (MUFA), which are typically seen in Mediterranean diets, are useful in lowering LDL-cholesterol without deleterious effects on HDL-cholesterol[184]. In addition the lower carbohydrate content decreases the post-prandial glucose surge and insulin response maintaining euglycaemic levels and reducing the risk of progression to insulin resistance and type 2 diabetes[144;313]. These observations are additional to improvements in insulin resistance due to weight loss[311].

Low GI and GL intake has been implicated to be protective in chronic diseases, but a meta-analysis of 37 prospective cohort studies concluded that the protection against heart disease or diabetes, attributed to such diets, was similar to that seen by increasing whole grain or high fibre intake and that the effect was not diet specific but as a result of the reduced surge in post-prandial glucose[51]. Another meta-analysis of 39 cohort or case-control studies examining the association of GL, GI and glucose loading to various neoplastic conditions, reported a protective benefit of these diets for colorectal and endometrial carcinomas, but not breast or pancreatic cancer[190]. Others have reported a risk reduction of up to 53% in breast cancer with low GL and GI diets[308;495]. Furthermore evidence suggests that the inclusion of 2 oily fish meals each week can help prevent cardiovascular disease through the effects of omega-3 polyunsaturated fatty acids[222], and inclusion of at least 5 servings of fruit and vegetables a day has been a widely accepted recommendation for cardiovascular disease and cancer prevention.

Low GL and GI diets have been reported to have favourable effects on cytokines and markers of sub-clinical inflammation seen in MS and obesity. The majority of studies available are either observational or cohort. In a cohort of 244 healthy females, dietary glycaemic load was significantly and positively associated with plasma hsCRP independent of all other conventional risk factors for ischaemic heart disease[331]. In the Health Professionals' Follow-up Study, adiponectin was identified to be low in individuals with high GL and low dietary fibre intake and

increased by up to 19% in those on high fibre, low GL diets[432]. Other studies have suggested that by reducing plasma free fatty acids, low GL diets may suppress the release of signalling inflammatory cytokines such as TNF-α and IL-6 from adipose tissue.

Indeed if low GL/GI diets prove to be effective in reducing markers of inflammation in addition to their benefit in weight loss, insulin resistance and dyslipidaemia, then it would be reasonable to use it for standard dietary recommendation instead of current advice.

3.7. The Mediterranean Diet

The Mediterranean diet is considered to be closely linked or even synonymous with low GL or GI diets. Its benefits were first highlighted by A Keys, when reporting on the Seven Countries Study, possibly the first to examine systematically the relation among lifestyle, diet, and the rates of heart attack and stroke in contrasting populations. Over 12,000 healthy men from different countries were studied. It was identified that the Greeks, despite a diet which consisted of 40% fat intake, had the lowest cholesterol levels and least reported cardiovascular events. Studying their diet further it was noted that most of the fat intake was derived from olive oil and olives. The remaining caloric intake came from cereals, vegetables and generally "blue fish"[6], with something of meat and derivatives, as well as a modest amount of wine. As most Mediterranean countries appeared to share similar dietary habits, the name "Mediterranean Diet" evolved (Table 17)[30;280].

An important part of the Mediterranean diet is the quality, not quantity, of fat intake. It encourages the ingestion of monounsaturated fats, found in seafood, olive oil and poultry, in preference to animal fats which are rich in the saturated form. Whether qualifying the type of fat within a diet has any benefits is yet to be fully proven. In one 28-day study individuals were randomised to diets high in fat intake, one arm using monounsaturated fats and the other saturated fats. Both groups lost equal weight, with triglycerides falling in both, but only significantly in the monounsaturated fat arm[89].

The benefits of the Mediterranean diet are very similar to those of a low GL diet, and for this thesis the two will be considered as one.

[6] Blue fish refers to sardines and anchovies

Table 17: Characteristics of the Mediterranean Diet

1.	High consumption of virgin olive oil.
2.	High intake of vegetables and fruits and legumes.
3.	Use of non-refined carbohydrates (portions to be adjusted to physical activity).
4.	Consumption of fish, specially oily (or “bluish”*) three or four times a week
5.	Consumption of milk and derivatives, cheese and yogurt (the original cheese was fresh goat cheese). Keep an eye on the saturated fats of the dairy products. Not too much!
6.	Three or four eggs per week.
7.	Moderate consumption of meat and saturated fats.
8.	One or two small glasses of wine a day, preferably red and at the main meals. White wine and beer are alternatives.
9.	Nuts as snacks

3.8. High Protein, High Fat, Low Carbohydrate

High-protein, high-fat, low-carbohydrate diets were the recommended therapy in the pre-insulin era. Towards the end of the twentieth century, the "popularity" of high-protein diets re-emerged but individuals received all sorts of messages about protein: when to eat it, how much to eat, what it will or won't do to blood glucose, and whether high intakes will or won't hasten the development of renal disease.

John Rollo was a Scottish Surgeon in the Royal Artillery who first recommended the use of a high-protein, low-fat diet for the treatment of diabetes in the pre-insulin era (1797) which he published in his "*An account of two cases of the diabetes mellitus".*

"Noon meal: Plain blood pudding, blood and suet only. Dinner meal: Game or old meats, fat and rancid old meats as fat as the stomach may bear."

—John Rollo's diet for Captain David Meredith, 1797[457]

The controversies which are associated with the benefits or hazards of a high protein intake have been documented as far back as 1929 when the Arctic explorer Vilhjalmur Stefansson who existed for seven years on a diet of only meat and fish (80% caloric intake from fat) was studied. Prior to this, a belief existed that a group of serious diseases were either caused directly by meat or prevented only by vegetables and therefore man was unable to exist on meat only. Stefansson's good health and tolerance of such a high protein and fat intake were suggested to be due to vigorous exercise and acclimatization to the extreme cold weather. Consequently, both Stefansson and Anderson (who had been in the Arctic with him) were placed on a 12 month meat diet while living within an urban environment. At the end of the trial, physical and metabolic assessments reported that neither suffered any ill effects in physical stamina, mental alertness, deterioration of renal function or hypocalcaemia[283;325;512].

With the rising problem of obesity low carbohydrate diets have increased in popularity with the most popular being the Atkins' Diet which was advocated by Dr R. C. Atkins, a cardiologist who himself lost weight following a low-starch diet studied by Alfred W. Pennington. He then successfully applied the same diet on a number of his overweight patients, and finally published it in his book "Dr Atkins' Diet Revolution" in 1972 where it rapidly became a bestseller. Dr Atkins followed his own dietary recommendations and it is of interest to know that when he underwent angiography in 2000 following a diagnosis of viral cardiomyopathy, his treating cardiologist reported that his coronary arteries were remarkably disease free despite other expectations from his dietary habits. Dr Atkins died in 2003 having sustained a head injury due to a fall. Many rumours have prevailed as to the cause of the fall with a cardiac arrest being implicated although never confirmed.

Atkins' diet is essentially based on the consumption of ≤50grams carbohydrate per day and involves several stages. It begins with a 2-week 'ketogenic induction' period, during which dieters consume less than 20grams of carbohydrate per day. Protein intake particularly in the form of red or white meats and eggs are encouraged with an unlimited allowance of fat. Within the induction phase not only are refined carbohydrates prohibited, but also fruits, bread, grains, starchy vegetables or dairy products other than cheese, cream or butter. Once the ketogenic induction stage is completed individuals will gradually increase their carbohydrate intake but should aim to maintain it at a level that promotes weight loss and borders on ketogenesis[40]. Such levels of carbohydrate restriction could vary between 25-100grams per day. The rationale behind ketogenic diets is that a reduced carbohydrate intake encourages the body to use alternative methods to produce energy (Figure 46, page265). Once running out of glycogen stores, insulin levels fall and the body turns to its fat stores in order to produce energy. Fatty acid oxidation produces ketones (acetone, acetoacetate, and β-hydroxybutyric acid) as its catabolic products which is the basis of the ketosis in these diets[485;486]. Following the success of the Atkins' diet several low carbohydrate diets were published including the "Carbohydrate Addict's Diet[231], the South Beach Diet[9] & the Protein Power Diet[131]"

The initial rapid weight loss seen with low carbohydrate diets is believed to be due to reduced caloric intake from appetite suppression secondary to the ketosis[366]

and the enhanced satiety from the increased protein intake[312]. Despite the *ad libitum* recommendation with Atkins' Diet, it is noted that the greatest weight loss is seen in those with the lowest caloric intake and the highest initial body weight[40]. A third cause for the initial weight loss is similar to most diets which is depletion of glycogen stores. Each gram of glycogen requires 3 grams of water for storage. This is consequently lost in the first weeks of dieting as glycogen is consumed in gluconeogenesis. Naturally once this process is complete, the rate of weight loss declines sharply[69]. Critics of such diets are quick to point out that this is weight that is rapidly replenished once dieting is over and carbohydrate intake is sufficient to replenish glycogen stores.

Speculation abounds with regards to the safety of ketotic diets. Many trials have been performed to try and assess their benefits and hazards. The general belief is that low carbohydrate diets are potentially nutritionally deficient lacking dietary fibre, vitamins, calcium, potassium, magnesium and iron due to the restrictions in food choices. Of note is that compliance with such diets is low and the drop-out rate much higher than for those diets with higher carbohydrate intake[367]. Reasons for dropping out tend to be for symptoms such as dehydration, headache, constipation and hypoglycaemia.

3.8.1. Benefits of Low Carbohydrate Diets

Weight loss attributed to low carbohydrate diets is reported to be greater than with other programmes. This appears to be true for the initial phase, but studies have suggested that at 12 months, the degree of weight loss achieved by individuals on a high or low carbohydrate regimen was similar[164;516]. In 2003 a systematic review of the safety of low carbohydrate diets from studies published between 1966 and 2003 concluded that among all published studies, participant weight loss while using low-CHO diets was principally associated with decreased caloric intake and increased diet duration but not with reduced carbohydrate content. They concluded that the evidence was scarce, and the duration of available trials was too short to make conclusive

recommendations[68]. Some observations have implied that weight loss with low carbohydrate, high protein diets was through reductions in body fat, whilst the higher protein intake, particularly the amino acid leucine, encouraged muscle protein synthesis[311].

Low carbohydrate diets are reported to positively improve many features of MS. In two randomised controlled trials, low carbohydrate diets compared to conventional low-fat, calorie-restricted diet, were superior in weight loss at 3 (6.8 vs. 2.7% of body weight) and 6 (7 vs. 3.2%) months. The significance was lost at 12 (4.4 vs. 2.5%) months. Improvements were noted in other markers of cardiovascular risk including reductions in triglycerides, increased HDL-cholesterol and improvements in insulin sensitivity[164;221;466]. Similar results were noted from a meta-analysis of 5 trials, including 447 individuals, comparing *ad libitum* low carbohydrate diets to low fat diets. Total and LDL-cholesterol were better improved by low fat diets and thus questions were raised querying which cardiovascular risk marker was most important to be targeted in individuals with MS[393]. Some have implied that the LDL particle size increases in low carbohydrate diets becoming less atherogenic[486]. A group studying ketogenesis for the treatment of epilepsy in 20 healthy individuals reported that polyunsaturated fatty acids exerted a more pronounced ketosis with less adverse effect on total or LDL-cholesterol as compared to saturated fat intake[169].

In studies looking at the effect of low carbohydrate intake on glucose levels, many have demonstrated reductions in post-prandial insulin response and stabilization of blood glucose levels. Some have implied that improvements in glycaemia is possibly linked to amino acid intake, particularly leucine, which plays a regulatory role in modulating skeletal muscle glucose oxidation and maintaining muscle mass in periods of energy restriction. This may contribute to the stabilization of glucose levels[311-313]. A study of 28 subjects with type 2 diabetes placed on a low carbohydrate diet for 16 weeks demonstrated that HbA1c improved from 7.5% to 6.3%. Only 21 individuals completed the study, but seven discontinued diabetes medications, and ten had dose reductions in hypoglycaemic drugs. The study was too short a duration to accurately reflect long term glycaemic control, but these results are promising and have been reflected in other trials[73;587].

Low carbohydrate diets positively affect blood pressure as reported in a study of 185 overweight individuals placed on a very low carbohydrate diet. A 15% decrease in weight reduced systolic (11.8mmHg) and diastolic (5.3mmHg) blood pressure over a period of approximately 17 weeks[88].

The evidence for the effect low carbohydrate diets have on markers of inflammation and cytokines is scanty. It seems that they may have beneficial effects reducing such markers as hsCRP, TNFα, interleukin-6, and intercellular adhesion molecule -1, as has been seen with other diets and weight loss. The effect seems to be a result of weight reduction and specific to the low carbohydrate intake[487].

One of the controversial health arguments raised against high-protein diets is their potential to cause renal dysfunction. Dietary recommendations for individuals with declining renal function are to reduce protein intake, so as to preserve eGFR. Even then the benefits of protein restriction on renal function have been unclear. Andrew Levey, reviewed data from the Modifications of Diet in Renal Disease (MDRD) trial and reported that the evidence was controversial and as a result felt it was difficult to supply any specific dietary recommendation[320]. In a study looking at protein intake with declining renal function Knight et al concluded that a high protein intake affected individuals with compromised renal function but certainly did not have an effect in those with normal kidneys[285]. Athletes have been recognised to partake of large amounts of protein for muscle building and certainly have not experienced secondary renal deficits[428].

In general studies suggest that low carbohydrate diets may be useful for cardiovascular risk protection. They reduce fasting insulin and glucose levels, improve blood pressure, and reverse atherogenic dyslipidaemia by increasing HDL-cholesterol, decreasing plasma triglycerides and possibly increasing LDL particle size. The mechanisms by which these effects occur are unclear but it is believed to be linked to the increased satiety from high protein intake, increased thermogenesis and more efficient energy expenditure[410].

3.9. Comparative Studies

3.9.1. Comparative Studies of the Effect of the Three Diets on Weight

The majority of studies comparing the superiority of the variable dietary interventions are of short duration, the longest being 3 years. Studies comparing an *ad libitum* low carbohydrate diet to a calorie restricted low fat diet, have demonstrated that weight loss is greater in the low carbohydrate groups by up to 50% or more[164;466;482;516;588]. Those studies which were of 12 month duration reported the changes to be sustained with additional benefits noted in lipid profile, and insulin resistance favouring the low carbohydrate group. One study did report that the weight loss was not sustained and after an initial reduction at 6 months of -7% in the low carbohydrate arm (low fat, -2.5%), weight was only -4.4% at 12 months (the low fat group remaining at -2.5%) [164]. A systematic review of 13 randomised controlled trials comparing low carbohydrate to standard recommendations reported the low carbohydrate groups to be superior in weight reduction at 6 and 12 months, although at 12 months the differences were small[237]. The superior weight reduction in addition to the cardiometabolic improvement implies that low carbohydrate diets may be better for cardiovascular risk reduction.

In a two year randomised controlled trial (DIRECT - Dietary Intervention Randomized Controlled Trial), 322 individuals were assigned to one of three diets, *ad libitum* low- carbohydrate, calorie restricted Mediterranean or calorie restricted, low-fat diet. Mean weight loss was greatest in the low carbohydrate and Mediterranean arms compared to the calorie-restricted low-fat arm, (4.7kg, 4.4kg and 2.9kg respectively). Changes in lipid profiles were similar to other trials with significant improvements in triglycerides, HDL-cholesterol and total cholesterol/HDL-cholesterol ratio seen in the low carbohydrate group. Changes in LDL-cholesterol were minimal and not significant in any of the groups. Improvements in glycaemic control were noted among those with diabetes in the Mediterranean arm but numbers (36) were too small to draw any conclusive results.

All three groups sustained reductions in blood pressure and waist measurements, but differences were not significant suggesting that these improvements might primarily be weight rather than diet related[483].

3.9.2. Comparative Studies on the Effect of the Three Diets on Insulin Resistance

Glycaemic control tends to improve with weight loss as insulin resistance improves. Although theoretically it seems reasonable to believe that low carbohydrate diets will be most effective at improving glycaemic control, evidence does not appear supportive. Suggestions were made that high protein intake could affect glucose tolerance based on Sweeney's observations when he demonstrated that individuals fed a high protein diet appeared to have an impaired response to an oral dextrose load as compared to those on a high carbohydrate diet[519]. Many other studies seemed to imply similar results suggesting that certain proteins may play an inhibitory role on glucose regulation[459;477]. However, it seems that this effect occurs with intravenous amino acid administration and has not been easily replicated with oral protein intake[179;311;395;396].

In a meta-analysis published in 1998 comparing the effects of high monounsaturated fat diets to high carbohydrate diets in subjects with type 2 diabetes, Garg et al was able to identify that the monounsaturated fat diet reduced insulin requirements, and stabilized fasting and post prandial glucose levels. No significant improvement in insulin sensitivity was established. The benefits could only be attributed to the reduced carbohydrate load[184]. More recent studies have more successfully demonstrated improvements in insulin sensitivity. In a crossover study 59 healthy individuals were placed on a saturated fat diet for 28 days then switched to either a low fat high carbohydrate regimen, or Mediterranean diet, for a further 28 days. Significant improvements in fasting insulin (32.2, 13.8 & 14.7IU/l respectively), overall glucose disposal (8.06, 6.61 & 6.25mmol/L respectively) and free fatty acids levels (0.52, 0.37 & 0.37mmol/L respectively) were seen in favour of

the Mediterranean and low fat diets[417]. In the KANWU study, 162 volunteers were assigned to an isocaloric high saturated or monounsaturated fat intake and followed for 3 months. At the end of the trial, insulin sensitivity was significantly impaired among the high saturated fat group (-12.5% vs. 8.8%). Changes in the lipid profile confirmed increases in Apo B, total-cholesterol and LDL-cholesterol (+2.1%, +2.5% & +4.1%respectively) among the saturated fat arm with reductions (-4.3%, -2.7%, & -5.2% respectively) in the monounsaturated group. Reductions in triglycerides and a rise in HDL-cholesterol were noted in both[558].

3.9.3. Comparative Studies on the Effect of the Three Diets on Lipid Profile

In the 1998 meta-analysis by Garg et al the superiority of a monounsaturated fat diet in improving lipid profile was demonstrated. Monounsaturated fat diets were accompanied by a significant lowering of serum triglycerides, total cholesterol, and VLDL-cholesterol levels, with increases in HDL-cholesterol. Although there was no improvement in LDL-cholesterol there were no evident deleterious changes[184]. A randomised controlled trial of 83 individuals assigned to a low fat, high monounsaturated fat or low carbohydrate diet confirmed similar results to most other trials. The low carbohydrate diet was more effective at reducing triglycerides and improving HDL-cholesterol. The authors concluded that there may be a role to use such diets in the short term to improve lipid profiles in individuals with dyslipidaemia[391]. A study of 53 overweight or obese healthy men and women randomised to a low-fat or moderate-fat diet for 10 weeks identified the moderate-fat diet to be superior by reducing triglycerides and the total cholesterol/HDL-cholesterol ratio. HDL-cholesterol remained unchanged. Despite similar weight loss in the low fat group, triglyceride levels increased, HDL-cholesterol decreased, and total cholesterol/HDL-cholesterol ratio remained static[416].

3.9.4. Comparative Studies on the Effect of the Three Diets on Inflammatory Markers

A randomised controlled trial of 40 overweight men and women with MS and atherogenic dyslipidaemia placed on either a low fat or low carbohydrate diet for 12 weeks, demonstrated similar reductions for hsCRP (-23%), vascular endothelial growth factor (-21%), vascular cell adhesion molecule 1 (-6%) and P-selectin (-11%) for both interventions. The low carbohydrate group was superior showing additional positive anti-inflammatory changes in TNF-α (−32 vs. −12%), the chemokines IL-8 (−33 vs. +4%) and monocyte chemo-attractant protein-1 (−24 vs. −5%), and the adhesion molecules E-selectin (−34 vs. −14%) and intercellular adhesion molecule 1 (−17 vs. −3%). PAI-1 levels reduced more in the low carbohydrate group (−34 vs. −8%) [162]. An analysis of 1,922 healthy females in the Nurses' Health Study noted that those adhering to a healthy dietary programme as assessed by the Alternate Healthy Eating Index had higher adiponectin levels (+24%), particularly of the active high molecular weight form and lower resistin levels (-16%) as compared to those with poor compliance. Additionally hsCRP (-41%), ferritin (-24%) and E-selectin (-19%) were lower in the same group of individuals, all results being adjusted for anthropometric measures and lifestyle factors[143].

In the DIRECT study, there were significant reductions in hsCRP among the Mediterranean-diet group (-21%) and low-carbohydrate group (-29%), during both weight-loss and weight-maintenance phases. High molecular weight adiponectin levels increased and serum leptin levels decreased with all forms of intervention without any inter-group differences, leading to the conclusion that these effects are particularly weight related and not an attribute of one particular diet[483].

4. CHAPTER 4: The Battle of the Bulge:-

A randomised study comparing the effect of three dietary approaches on cardiovascular risk in subjects with the metabolic syndrome -

4.1. Introduction & Rationale

The epidemics of obesity and metabolic syndrome are increasing with substantial CVD risk. That insulin resistance, hyperinsulinaemia, dyslipidaemia, and obesity collectively form the metabolic syndrome has been discussed extensively in the previous chapters[273;510]. Obesity particularly appears to play a significant role and is a major factor in the development of the others[373;379].

Obesity, insulin resistance, dyslipidaemia and hypertension have all been linked to elevated levels of cytokines that are associated with atherogenesis [63;435]. The raised inflammatory cytokines associated with diabetes, metabolic syndrome and obesity are very similar suggesting that they have one and the same cause, a surfeit of fat. These include TNF-α, adiponectin, leptin, resistin, PAI-1, and angiotensin which are thought to be partially responsible for the development of insulin resistance by inhibiting insulin action [342;490;491].

Adiponectin is produced exclusively by white adipose tissue and appears to have a central role in the metabolic syndrome, in addition to anti-atherogenic and anti-inflammatory effects [484]. Plasma adiponectin levels are reduced in obesity and type 2 diabetes [342;484], hypertension, dyslipidaemia or hyperglycaemia all of which are components of the metabolic syndrome [484]. Studies have demonstrated an inverse correlation between plasma adiponectin concentrations and the severity of insulin resistance [342].

Leptin, synthesised and secreted mainly from adipose tissue, is an afferent signal molecule that interacts with the appetite and satiety centres in the brain to regulate body weight [490]. This hormone contributes to the regulation of both food

intake and energy expenditure [12], by enhancing thermogenesis and metabolic rate [490]. Animals that are defective in either leptin synthesis or leptin receptor function become obese and develop hyperinsulinaemia and insulin resistance [490].

Resistin, has been found to impair glucose tolerance and insulin action when administered to obese mice [342;490]. Human studies correlate it with other inflammatory markers, insulin resistance and increasing adiposity suggesting that resistin may serve as a link between obesity and insulin resistance [171;342;376].

PAI-1 is an inhibitor of plasminogen activators and inhibits fibrinolytic activity [490]. It is mainly synthesised by endothelial cells, liver and recently adipose tissue[490]. Plasma concentration of PAI-1 is elevated in subjects with type 2 diabetes and in humans there is a direct correlation between the amount of visceral fat and plasma levels of PAI-1 [490] although the mechanisms linking these together remain unclear.

It is this association between obesity and metabolic complications that constitutes the main argument justifying weight reduction programmes which, if successful, can lead to a substantial improvement in the metabolic profile of the obese[573] or even normalisation[538]. Despite 35% of woman and 45% of men in the USA, at any given time professing to be "on a diet", the prevalence of obesity has doubled in the past 20 years suggesting that inadequate dietary advice may be a contributing factor[164].

The conventional recommended dietary approach to weight management, is a low-fat, high-carbohydrate, energy-deficient diet (30% energy from fat, 10-15% protein, 55-60% carbohydrate) [60;164;338]. Its suitability is being challenged with increasing evidence stating that conventional diets may promote weight gain, raise plasma triacylglycerols, affect LDL-cholesterol, reduce body fat oxidation and satiety[312;486]. Consequently, low-carbohydrate, high-protein, high-fat diets, such as the Atkins diet, have become increasingly popular with results comparing them favourably to conventional diets, particularly for weight loss, at least in the short term[164;466].

The quality of carbohydrate consumed is believed to be an important factor in the success of low carbohydrate diets as individuals are encouraged to choose options low in glycaemic load. Carbohydrate replacement in low fat diets is typically of a high glycaemic index which increases inflammatory markers that promote atherothrombosis and hyperinsulinaemia [165;331].

In contrast low GL diets are beneficial in improving lipid profiles and glycaemic control, and reducing cardiovascular risk. A higher protein intake has been demonstrated to improve HDL-cholesterol to triglyceride ratios and promote satiety. Diets rich in monounsaturated fatty acids, are proven to reduce LDL-cholesterol, without negatively affecting HDL-cholesterol[67;184;312;332]. Evidence available supports the inclusion of 2 oily fish meals each week as a source of omega-3 polyunsaturated fatty acids, and the inclusion of at least 5 servings of fruit and vegetables a day as a means of cardiovascular disease and cancer prevention[222].

Despite dietary intervention being a well-established primary management plan for obesity and MS, the evidence about which diets may beneficially modify classical and contemporary CVD risk factors is limited. The work to be described examines the effects of three different dietary approaches on weight loss achieved and possible changes, potentially beneficial or otherwise, of certain CVD risk factors. This information may potentially help to clarify some of the mysteries surrounding the metabolic syndrome, and help reduce its progression to type 2 diabetes.

The first of the diets used in the study is based on the conventional high carbohydrate, low fat, calorie-restricted diet currently advised to most individuals. This will be compared with a second diet of low carbohydrate, high fat, high protein ad libitum diet (like Atkins) and with a third diet based on the Mediterranean diet. Details of the diets are in Table 18.

It is expected that the metabolic diet will have a clinical desirable effect on key risk factors for cardiovascular disease including insulin resistance, plasma lipid profile and inflammatory markers.

Table 18 : The three proposed dietary plans and expected metabolic effects

	High carbohydrate, Low fat (Conventional)	**Low carbohydrate, high fat/protein (Atkins)**	**Proposed dietary regimen (Metabolic Plan)**
Dietary formulation	50-60% CHO 10-15% protein <30% fat <10% sat fat 20% poly/mono	10-12% CHO 30-40% protein 50-60% fat 15-20% sat fat 15-20% mono fat 15-20% poly/mono	40% CHO (low GI) 25% protein 35% fat <10% sat 15% mono 10% poly – 5% omega 3. 5 servings of fruit and vegetables a day and 2 oily fish meals each week
Expected metabolic effects of each diet	LDL-cholesterol decrease HDL-cholesterol decrease Triglyceride decrease Insulin sensitivity improves in relation to weight loss	LDL-cholesterol static/ minimal decrease HDL-cholesterol increase Triglyceride decrease. Insulin sensitivity improves in relation to weight loss	LDL-cholesterol decrease HDL-cholesterol increase Triglyceride decrease Insulin sensitivity greater than expected for weight loss

4.2. Inclusion and exclusion criteria

The study aimed to recruit 120 medically fit subjects, aged between 18–70 years, who fulfilled the NCEP-ATP III criteria for MS to take part in a 12 month dietary intervention randomized controlled trial. To reduce the potential for external influences to produce any significant effect**,** individuals with uncontrolled diabetes, or were on insulin, weight modifying agents, or lipid-modifying drugs were excluded. In view of the concerns of the effect of high protein diets on renal function those with renal impairment or long term chronic medical problems that may affect renal function or restrict their diet were excluded.

A comprehensive list of inclusion and exclusion criteria is given below.

Inclusion criteria

- Males or non-pregnant females fulfilling the criteria for the metabolic syndrome. Criteria for metabolic syndrome used for recruitment included the presence of 3 or more of the following criteria -
    1. Abdominal obesity (waist circumference >102 cm in men and >88cm in women). As all those recruited were Caucasian no modifications were necessary
    2. Triglyceride level >150mg/dL (1.7mmol/l)
    3. HDL-cholesterol <40mg/dL (1.03mmol/l) for men and <50mg/dL (1.3mmol/l) for women
    4. Blood pressure >130mmHg systolic or >85mmHg diastolic
    5. Fasting plasma glucose > 110mg/dL (6.1mmol/dL)*[7]
- Age 18 – 70 years
- If on treatment for hypertension, must be stable on hypertensive therapy for past 3 months
- Type 2 diabetes on diet, metformin or sulphonylureas, must be stable on diabetes medication for past 3 months and have HbA1c <10%
- Willing to be randomised to any of three diets

[7] If the subject had type 2 diabetes, they were considered to fulfil the fasting glucose criteria regardless of their fasting glucose level.

Exclusion criteria

- Recent cardiovascular or thromboembolic event (i.e. stroke, myocardial infarction, or pulmonary embolism) within past 4 months
- On-going chronic inflammatory condition – vasculitis, chronic ulcers (feet, skin), inflammatory bowel disease, malignant disorder
- HbA1c $\geq$ 10%
- Renal impairment (Creatinine > 135umol/l)
- Hepatic impairment. (ALT or AST > 4 times upper limit of normal)
- On lipid-modifying therapy
- Poorly controlled diabetes (requiring regular and recent changes to diabetes medication within the past 3 months)
- Type 1 or Type 2 diabetes on insulin
- Type 2 diabetes on a thiazolidinedione (rosiglitazone*[8]/pioglitazone).
- Untreated thyroid disorders – should be stable on thyroid modifying medication for at least 3 months
- Known Cushing's disease/syndrome
- On an anorectic agent (i.e. orlistat, sibutramine*) – can be given a 4 week washout period.
- Food allergies/intolerance
- Vegetarian
- Unwilling to be randomised to one of three diets

[8] Rosiglitazone and sibutramine had been available at the inception of the protocol although they were later withdrawn from use.

5. CHAPTER 5: Study Methodology

5.1. Ethical Consideration:

Ethical approval was obtained from the Bath Local Research Ethics Committee which was in partnership with the Bristol Local Research Ethics Committee and therefore granted approval for individuals to be approached in both localities as an overlap existed. The main ethical issues included: informed consent, autonomy to participate in or withdraw from the study, causing no harm to the participants, anonymity and data confidentiality. All participants gave informed verbal assent and written consent to participation in the study prior to any form of testing. Participants were verbally informed of the nature and reason for each procedure, and were given an opportunity to ask questions. Participants were also reassured that they did not have to give any reason for withholding from participating in the testing procedures or withdrawing from the study.

5.2. Recruitment Procedure:

Letters were sent out to GP practices requesting permission to run a search looking for suitable subjects (Figure 42: GP Invitation Letter, page252). Once approval was given by the practices, subjects fulfilling the criteria for metabolic syndrome were identified from the Department of Diabetes & Endocrinology diabetes database (DIAMOND, Hicom, Surrey) at the Royal United Hospital, and GP database searches. Database interrogation involved running several searches looking for all patients with type 2 diabetes or fasting glucose greater than 6.1mmol, hypertension or systolic blood pressure greater than 140mmHg, HDL-cholesterol less 1mmol/l and triglycerides greater than 1.8mmol/l. The search was asked to exclude patients with a BMI less than 24kg/m^2, an HbA1c greater than 10%, and individuals on statins, or insulin.

Once the initial list was generated, the search was then modified to exclude all individuals who did not have at least two of the criteria for metabolic syndrome. The list generated for the second search was then individually reviewed going through blood results available on the Royal United Hospital's WebIce (Intercontinental Exchange, Inc) programme. All with fluctuating readings for HbA1c or thyroid function, biochemical evidence of renal or hepatic dysfunction were then omitted from a final list of potential candidates.

A letter of invitation explaining the study was sent out to the potential participants (Figure 43: Patient Invitation Letter, page254). Enclosed was a form which volunteers completed indicating their interest, or lack of, in the study (Figure 44: Initial details form, page255). If they wished to be considered for the study, if eligible, then they would complete the details form including the section for contact details, known medical history, and a list of current medications and return it in the stamped envelope which was provided.

The patients who did not fulfil study criteria due to medical or drug history were written to with an apology, explaining the reason for their unsuitability. Those whom on paper appeared to potentially fulfill the basic requirements were invited to the Wolfson Centre, Royal United Hospital, Bath, for an initial screening visit. Before attending they were asked to complete a baseline food questionnaire which they brought to the interview (Table 53: Baseline Dietary Questionnaire 42: Baseline Dietary Questionnaire, page257).

5.2.1. The Screening Visit:

At the screening visit, interested participants were invited to attend the research centre, fasting (12 hours) where the study was discussed in detail. If still willing to proceed they were then asked to sign a consent form (Figure 45: Consent Form, page256), prior to further interventions. Waist and weight measurements, blood pressure and baseline fasting bloods were taken to ensure they fulfilled the biochemical criteria for the study, followed a physical examination. If blood pressure

was high recommendations for antihypertensive therapy adjustments were made and only when readings were stable for more than three months were they re-screened*[9]. Each qualifying subject was given a set of weighing scales and asked to carry out seven consecutive days of a weighed food diary.

Qualifying subjects were booked to return in a fortnight for the randomization visit. During this time they were to continue with their normal dietary habits and make no modifications. Those who failed to meet the criteria for MS were informed of the reasons and provided with dietary and lifestyle advice as a thank you

5.2.2. The Randomization Visit:

Subjects would attend having fasted for twelve hours. They would have measurements taken including weight, height, waist circumference, blood pressure and baseline bloods for measurement of renal function, fasting lipid profile, fasting glucose, and other analytes as indicated below. They were then given a 75gram glucose load to drink followed by a 2 hour glucose sample to complete a glucose tolerance test.

A diet was then allocated by the patient choosing a number from a spreadsheet ranged from 1 to 120. Each number had been randomly allocated to one of the three dietary categories by an independent third party who held the allocation coding. Once randomized the subject would be counseled by the investigator or a trained research dietician. The research dietician was available for five sessions per week. She would see subjects on a regular basis alternating the sessions with the researcher if possible providing dietary advice, and counseling for those who were particularly struggling with lack of weight loss or dietary compliance issues. All subjects would be seen by the medical researcher for their initial visit, randomization and each visit where blood taking or an examination was required.

[9] *Two candidates who were persistently keen in taking part insisted on being re-screened once their blood pressure had been stabilized by their doctor.

Those allocated to **Low-Fat Diet** were counselled to consume low-fat grains, vegetables, fruits, and legumes and to limit intake of added fats, sweets, and high-fat snacks. The target macronutrient composition was 55% of energy from carbohydrate, 25% from fat, and 15% from protein. The intervention was not designed to reduce dietary glycaemic index and glycaemic load; rather, the aim was to prescribe a diet consistent with the current high carbohydrate, low-fat guidelines. The individuals were given a calorie allocation according to their weight, and level of physical activity with a calculated 500kcal deficit which is compatible with an average 0.5kg weekly weight loss Table 55: Table of Prescribed Energy Intake, page263). They were supplied with a portion conversion guide (Figure 48: Copy of the eating plan given to those on a HC diet, p276) and a seven day food menu to help facilitate caloric estimation for most general foods.

The subjects who were placed on the **Metabolic Plan (Low–Glycaemic Load) Diet** were counselled to consume low–glycaemic load foods (particularly non-starchy vegetables, legumes, and fruits) and to limit intake of high–glycaemic load foods (such as refined grains, starchy vegetables, fruit juices, and sweets). Attention also was directed toward consuming "healthy fat" high in mono-unsaturated fatty acids, such as those particularly found in shellfish, oily fish, nuts, seeds, and rapeseed or olive oils. The target macronutrient composition was 40% of energy from carbohydrate, emphasizing low-glycaemic index sources, 35% from fat, and 25% from protein. Participants were equipped with food-choice lists that delineated products into low- or high-glycaemic load. Again subjects were given a diet calculated to provide a daily 500kcal deficit, and provided with food charts (Figure 47: Copy of the eating plan given to those on the LGL Diet, page270) and sample menus to aid them in their daily allocations.

Subjects allocated to the **Atkins' Diet** were counselled to a high fat, high protein diet with an initial carbohydrate restriction of 20grams per day. They were also supplied with the Atkins' diet revolution book and asked to follow its instructions. The 20gm carbohydrate restriction was to be maintained for the first two weeks followed by a gradual increase in carbohydrate intake as per recommendations (Figure 46: The four phases of Atkins, page265). The carbohydrate introduced was encouraged to be of a low glycaemic load in the form of non-starchy vegetables and fruits (Table 56: List of allowed foods in Atkins adapted from [40]). As there had

been substantial speculation on possible mineral and vitamin deficiency associated with Atkins' diet, all were supplied with a daily calcium and vitamin D tablet and a daily multivitamin/mineral tablet.

5.2.3. The Follow-up Visits: -

Once randomised subjects were given an initial two weeks trial run, to see if they were able to maintain the restrictions placed on them by their dietary allocation and to ensure that they did not suffer any adverse effects. If they were able to complete the two weeks they would then be considered to be in the study and their progress monitored on a regular basis.

Having completed the run in period and consented to continue on the trial, subjects were seen fortnightly for the first twelve weeks. At each visit they had their weight and waist circumference measured, and received dietary counselling.

At week six in addition to anthropometric measures, individuals were given a four day weighed food diary to complete and visits continued on a fortnightly basis.

At the next major visit (week 12) participants attended fasting. Blood was taken for renal function, lipid profile, glucose, glycosylated haemoglobin (if they had type 2 diabetes) and other analytes. This is the stage where subjects were assumed to move from a fast weight loss phase to a slower but steady rate prior to moving into a maintenance phase. Weight, waist and blood pressure measurements were taken and calorie requirements readjusted according to an individual's weight and energy requirements. At the visit, subjects were given forms to complete another three day weighed food diary. One of the days was to include a weekend day, to give a general overall picture.

From this point visits were changed to a 4 weekly basis with subjects calling in the interim should they have any queries or require extra advice. Visits at week 16,

and 20 were similar to the initial fortnightly visits with weight and waist measurements and dietary counselling.

The next full visit was at week 24 where subjects were re-measured for weight, waist and blood pressure, again provided fasting blood samples and a repeat glucose tolerance test, and caloric requirements were readjusted as required. Most of those on the Atkins' diet had by now reached a maintenance phase with an intake of approximately 50-70grams of carbohydrate. Individuals were counselled and encouraged to maintain carbohydrate of a low glycaemic load quality, and asked to complete another three day weighed food diary. At this stage, subjects were believed to be entering into a phase where weight loss would be slow or there would be weight-loss maintenance. This was discussed with each individual to avoid disappointment and encourage compliance. Calorie changes according to weight and activity requirements were made, and individuals re-educated on their specific diet plan.

Follow-up visits continued on a 4 weekly basis with weight and waist measurements taken at each visit, and dietary counselling provided for those who required it.

The penultimate visit was at week 48. Participants attended fasting overnight. Bloods were taken as on previous visits and a glucose tolerance test performed. Weight, waist and blood pressure measurements were recorded, and a physical examination was performed. Individuals were given a final four day weighed food diary to complete.

They returned for their final visit a fortnight later. At this visit they handed in their scales and final food diaries. The results of their weight, blood pressure and general laboratory bloods were discussed. Changes were made to the diet to help each individual to continue on a regimen most suited to that person and to encourage weight-loss maintenance.

At all stages of the study, a healthy life-style was encouraged, but at no point was an exercise regimen ever prescribed.

5.3. Data Collection

5.3.1. Anthropometric variables

Height was measured at the start of the study, while weight and waist measurements took place at each visit. Measurements for height were taken with candidates standing straight, heels placed together using a wall mounted stadiometer. Subjects were asked to gaze straight ahead with the back flat against the wall as the headboard was lowered to the most superior point of the head with enough pressure to compress the hair. Measurements were taken to the nearest .01m.

Weight measurements were collected with candidates dressed in light day clothes to the nearest 0.1kg using calibrated electronic scales (Tanita Corp, Tokyo, Japan).

Waist circumference was assessed at the mid-point between the lowest rib and the iliac crest and at the iliac crest as recommended by the IDF[13].Waist circumferences are reported to the nearest 0.1cm using an inflexible tape measure.

BMI was calculated as body mass/ height^2, where body mass is expressed in kilograms (kg) and height in metres (m).

5.3.2. Blood pressure

Blood pressure was measured using a digital OMRON (Omron HEM-773AC, UK) blood pressure machine. 3 readings were taken after the subject had been sitting quietly for 5 minutes and the average of the last two was used as the blood pressure reading. The appropriate cuff size was used in all measurements and readings were taken either by the researcher or a dedicated research nurse. Blood pressure measurements were completed before blood sampling.

Processing of blood samples

When subjects attended on their fasting visits (12 – 13 hours), 75mls of blood was drawn into appropriate BD Vacutainer™ tubes by venepuncture using Butterfly needles "21g" (BD Vacutainer® Safety-Lok™ Blood Collection Set ™) aided by the use of a tourniquet. Blood withdrawal was undertaken by the researcher or a dedicated research nurse who were experienced at phlebotomy.

Sample collections were 10mls for:-

- A **serum separation tube:** urea and electrolytes, liver functions, and fasting lipid profile
- A **2-ethylenediaminetetraacetic acid (EDTA) tube**: full blood count and glycosylated haemoglobin if the subject had diabetes
- An **heparinised tube:** coagulation screen and fibrinogen
- A **fluoride oxalate tube:** fasting glucose

These were analysed at the Central Laboratories, Pathology Department, at the Royal United Hospital as part of routine clinical samples. Results for lipids and glucose were incorporated into the data. The remaining 60mls was taken as 40mls in four serum separation tubes and 25mls in three EDTA tubes. All tubes were inverted 8 - 10 times to ensure proper mixing. EDTA tubes were immediately spun at 4˚C for 20mins at 1000g in the centrifuge. Serum separation tubes were placed in a 4˚C fridge for 20 minutes to allow for clotting and then spun down at 4˚C at 1000g for 20mins.

Serum and EDTA tubes were aliquotted into individual safe lock tubes and stored in a -70°C freezer until study end. The samples were thawed in batches to run enzyme-linked immunosorbent assays (ELISA) under similar circumstances and reduce the margin for error.

The aliquots were divided as such:

The EDTA samples were intended to be used for: -

- 5ml for lipoprotein subfractions
- 1ml for HDL-cholesterol
- 4 x 500ul samples for endothelin, CRP, visfatin and RBP-4
- 4 x 50ul samples for PAI-1, hsCRP and measuring oxidative stress
- 2 x 40ul samples for electrophoresis

The serum samples were intended to be used for:-

- 8 x 500ul samples for RBP-4, visfatin, TNFα, IL-1β, oxidised LDL, and MCP-1
- 4 x 300ul samples for IL-6 and IL-8
- 6 x 150ul samples for leptin, resistin, and adiponectin
- 4 x 60ul samples for insulin
- 5 x 50ul samples for leptin, resistin, and adiponectin
- 4 x 10ul samples for leptin, resistin, and adiponectin

As at the start of the study it had not yet been identified which ELISA kits would be used it was felt best to divide the serum and plasma into numerous safe lock tubes allowing for plenty of spares and to avoid repetitive defrosting and sample degradation.

5.3.3. Biochemical analyses

Insulin, hsCRP, and the adipocytokines were run in batches using ELISAs. Sets were chosen depending on the assay sensitivities, company support, and reliability from previous experience.

Consequently ELISA kits were sourced from three different companies.

- **AdipoGen Inc., Adipogen Institute for Life Science, Seoul, Korea**: for RBP-4 and visfatin kits
- **DRG Instruments GmbH, Marburg, Germany**: for insulin and leptin
- **R&D Systems, Inc., Minneapolis, USA**: for PAI-1, adiponectin, hsCRP, and resistin.

The ELISAs were run by Dr Julia Reid, clinical research scientist at the Wolfson Centre, Royal United Hospital

5.3.3.1. ELISA Techniques

Insulin ELISA

Serum insulin analysis was performed using a DRG Insulin Enzyme Immunoassay Kit. This is a solid phase enzyme-linked immunosorbent assay (ELISA) which utilises chemiluminescent technology and is based on the sandwich principle which utilises two antibodies (capture antibody and detection antibody) to provide the "sandwich".

The ELISA kits contained:

1. The **Microtitre wells** (96 as 8 x 12) which were already pre-coated with anti-Insulin monoclonal antibody **(capture)**
2. **Zero Standard solution**
3. **Standard (Standard 1-5) solution**
4. **Enzyme Conjugate** : mouse monoclonal anti-Insulin antibody conjugated to biotin
5. **Enzyme Complex (detection)** : Streptavidin HRP Complex
6. **Substrate Solution**: Tetramethylbenzidine (TMB)
7. **Stop Solution**: 0.5M H_2SO_4,
8. **Wash Solution**

Serum samples (60µl aliquots) were retrieved from the -70°C freezer and allowed to thaw to room temperature prior to use.

25 µl of standard solution or serum sample were aliquotted into the wells with the standard ones identified to be used as a reference point. 25 µl of enzyme conjugate (mouse monoclonal anti-Insulin antibody conjugated to biotin) was then added and mixed thoroughly allowing for the two to react and bind. The mixture was incubated for 30 minutes at room temperature and then excess contents were removed prior to

washing. Each well was washed three times with 400ul wash solution supplied in the kit to remove any unbound conjugate.

Fifty microliters of enzyme complex (Streptavidin HRP Complex) was added to each well and incubated at room temperature for a further 30 minutes allowing it to bind to the biotin-anti-Insulin antibody. The degree of binding should be directly proportional to the sample's insulin concentration. The wells are then once more washed three times with 400ul of wash solution followed by the addition of 50µl of substrate solution and a further 15 minute incubation at room temperature during which each well should have fully developed. To stop the reaction 50µl of Stop Solution was added to each well. Depending on the underlying insulin concentration each well will have developed to produce an equivalent degree of colour intensity which was then quantified by the use of microtitre plate reader.

All the ELISAs used facilitated the sandwich method for substrate extraction and analysis. The length of incubation periods and substrates differed and are recorded in the table below (Table 19& Table 20). For all protocols plates would be carefully and thoroughly washed with the washer solution between incubation periods. Samples were completed in duplicate and readings were taken as the average of the two. Any outlying results would be repeated to ensure accuracy. All readings were taken with the use of a microtitre reader able to read at 450nm.

Table 19: A list of ELISA protocols undertaken

	First Incubation	Second Incubation	Third Incubation	Fourth Incubation	Sensitivity
DRG Instruments GmbH					
Insulin	Wells pre-coated with anti-Insulin monoclonal antibody. 25µl substrate + 25µl mouse monoclonal anti-insulin antibody conjugated to biotin Incubate for 30 minutes	50µl Streptavidin HRP Complex Incubate for 30	50µl of TMB Incubate for 15 minutes		analytical sensitivity 1.76IU/mL intra-assay variability 2.6%. inter-assay variability 2.9%
Leptin	Wells pre-coated with monoclonal anti-Leptin antibody 15µl substrate + 100µl Assay buffer Incubate for 120 minutes	100µl polyclonal Leptin antiserum Incubate for 30 minutes	100µl anti-rabbit peroxidase conjugated to horseradish Incubate for 30 minutes	100 µl of TMB Incubate for 15 minutes	analytical sensitivity 1.0 ng/ml. intra-assay variability 5.9%. inter-assay variability 11.55%
AdipoGen Inc					
Retinol Binding Protein 4	Wells pre-coated with recombinant human RBP4 50µl substrate + 50 µl polyclonal antibody against human RBP4 Incubate for 60 minutes	100µl HRP conjugated anti-rabbit IgG Incubate for 60 minutes	100 µl of Substrate Solution (chromogenic reagents) Incubate for 20 minutes		analytical sensitivity 1.0 ng/ml. intra-assay variability 2.6%. inter-assay variability 3.5%
Visfatin	Wells pre-coated with monoclonal antibody against human visfatin Add 100µl substrate Incubate for 180 minutes	100µl polyclonal Ab against human visfatin Incubate for 60 minutes	100µl HRP conjugated anti-rabbit IgG Incubate for 60 minutes	100 µl of Substrate (chromogenic reagents) Incubate for 10 minutes	analytical sensitivity 30pg/ml. intra-assay variability 3.8%. inter-assay variability 8.2%

Table 20: A list of ELISA protocols undertaken, continued

	First Incubation	Second Incubation	Third Incubation	Fourth Incubation	Sensitivity
R&D Systems					
Adiponectin	Wells pre-coated with a mouse monoclonal antibody against HMW Adiponectin 100 µL of Assay Diluent + 50µl of substrate Incubate for 180 minutes	200µl of HMW Adiponectin Conjugate Incubate for 60 minutes	200 µL of Substrate Solution (TMB + $H^2O^{2)}$ Incubate for 30 minutes		analytical sensitivity 0.195 ng/mL intra-assay variability 2.6%. inter-assay variability 8.6%
PAI -1	Wells pre-coated with a mouse monoclonal antibody against PAI-1 50µl substrate + 50 µl assay diluent Incubate for 120 minutes	200 µl of Serpin E1/PAI-1 Conjugate Incubate for 120 minutes	200 µL of Substrate Solution (TMB + $H^2O^{2)}$ Incubate for 30 minutes		analytical sensitivity 0.026 ng/mL intra-assay variability 4.4%. inter-assay variability 9.5%
Resistin	Wells pre-coated with a mouse monoclonal antibody against Resistin 100 µL of Assay Diluent + 100µl of substrate Incubate for 120 minutes	200µl of Resistin conjugate Incubate for 120 minutes	200 µL of Substrate Solution (TMB + H2O2) Incubate for 30 minutes		analytical sensitivity 0.059 ng/mL. intra-assay variability 5%. inter-assay variability 8.2%
Hs CRP	Wells pre-coated with a mouse monoclonal antibody against CRP. 100 µL of Assay Diluent + 50µl of substrate Incubate for 120 minutes	200 µL of CRP Conjugate Incubate for 120 minutes	200 µL of Substrate Solution (TMB + H2O2) Incubate for 30 minutes		analytical sensitivity 0.010 ng/mL. intra-assay variability 4.4%. inter-assay variability 6%

5.3.4. Calculating Non-HDL-Cholesterol

Non-HDL-Cholesterol was calculated by subtracting HDL-cholesterol from total-cholesterol measurements. Measurements were recorded in mmol/l.

5.3.5. Cardiovascular Risk Calculation

10 year predictions of CVD and CHD risks were calculated using the University of Edinburgh Cardiovascular Risk Calculator which is based on the Joint British Societies' (JBS2) Cardiovascular Disease Risk Prediction charts (Figure 49, page282). This is an on-line calculator into which the individual's age, sex, smoking status, systolic blood pressure, total and HDL-cholesterol values can be entered to give a prediction of their risk for experiencing a cardiac or cardiovascular event within the following 10 years. Individuals who have a greater than 20% risk for developing CV disease should be initiated onto statin therapy according to the current lipid management guidelines [462].

5.3.6. Calculation of Insulin Resistance

Insulin resistance and sensitivity were calculated using the Homeostasis Model Assessment (HOMA) calculator downloaded from the University of Oxford website (http://www.dtu.ox.ac.uk/homacalculator/index.php). The HOMA calculator provides an estimate of beta cell function (%B) and insulin sensitivity (%S) through a mathematical equation that is based on a theory that fasting plasma insulin and glucose are regulated by a hepatic-beta cell feedback loop and accounts for variation in insulin and glucose secretion[124;356]. Insulin resistance (HOMA IR), is provided through calculating the reciprocal of insulin sensitivity (100/%S). The gold standard

for measuring insulin resistance is considered to be through the use of insulin clamps, but these studies are lengthy and would have been difficult to perform on the number of individuals who took part in this study.

The HOMA calculator at the time would only accept insulin levels in SI units (pmol/L) and all insulin levels needed to be converted from µIU/ml to pmol/L by multiplying by 6.945[460]. Plasma glucose (mmol/l) and insulin levels were inputted into the calculator and the results for insulin resistance and sensitivity were tabulated.

5.4. Statistical Analysis

Data were tabulated and stored on a Microsoft Access database, copied to Microsoft Excel for graphs and basic calculations and to IBM's Statistical Product and Service Solutions (SPSS version 14 and then 18) programme for statistical analysis.

Subjects included in the final analysis were those who had completed to the end of the 12 month study period although an intention to treat analysis for weight loss was run to compare differences in outcomes.

Sample size was calculated using IBM SPSS SamplePower 3 Programme. Initial calculations were based on the differences expected between the High Carbohydrate, Low Fat (control) and the High Fat Low Carbohydrate (Atkins') group. In order to achieve a power of 95%, it was expected that 33 individuals be recruited in to each arm. The calculations took into account that there would be a 25% drop-out rate with average weight loss in the control group being 4kg while that in the other group 8kg. The alpha was set to be two-tailed at 0.01.

Power calculation of the study was 0.99. This was based on 40 subjects per category and the effect size (*f)* calculated to be 0.5 on the basis of the mean expected weight loss for each category.

Analysis of variance (ANOVA) was used to compare the differences in results between the three groups at each of the study time intervals. Post hoc multiple pairwise comparisons among the three diet groups were performed with both Bonferroni and Dunnett testing. Differences were only considered significant should p-values for both be $p < 0.05$.

Within group changes comparing mean differences at baseline to each study interval point was by means of the paired *student t-test,* which tested for parametric data. Correlations between the continuous data variables was assessed using Pearson correlation coefficients.

Results were taken to be statistically significant for *p*-value of <0.05 (two-tailed), corrected for multiple comparisons.

From here on while presenting and discussing the results, the three dietary groups will be mentioned as follows-

- High Fat Low Carbohydrate (Atkins') as **Low Carbohydrate or LCD**
- High Carbohydrate, Low Fat (Control) as **Low Fat or LFD**
- Low Glycaemic Load (Mediterranean) as **Low Glycaemic Load or LGL**

6. CHAPTER 6: The Effects of the Three Dietary Interventions on Weight, Blood Pressure and Lipids

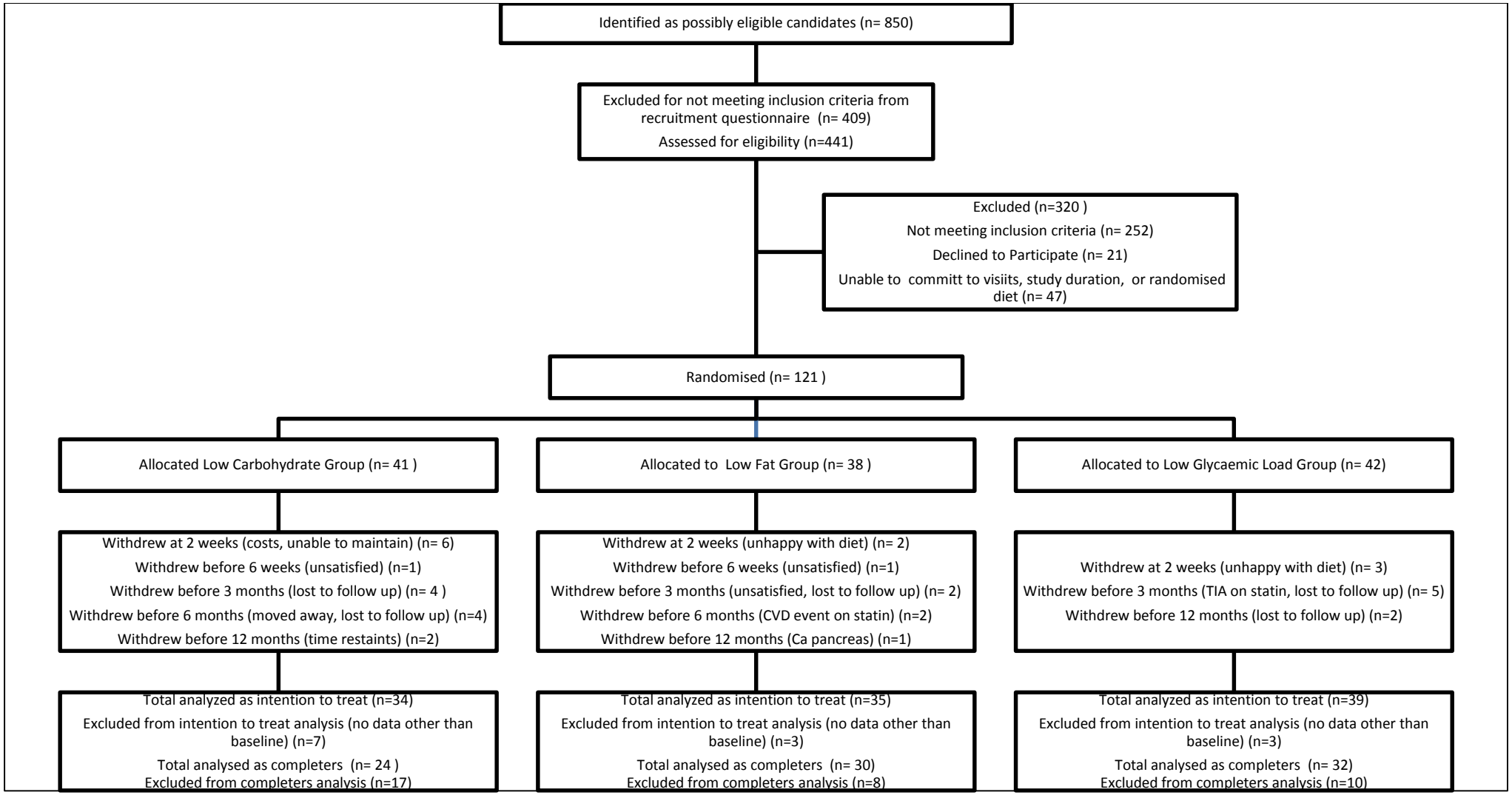
6.1. Subjects

850 subjects were identified as being potentially suitable to take part and were invited, by letter with a questionnaire attached, to take part in the study (Figure 43). Over 400 responded and sent in the completed questionnaire. 184 individuals attended for initial screening and of these 121 fulfilled the study criteria and were randomised to a dietary intervention. 11 withdrew within the first two weeks citing disappointment with dietary allocation, inability to maintain diet due to financial or disciplinary restraints or other unspecified reasons. Two more dropped out prior to completing 6 weeks. Only individuals who completed the study period (86) were included in the final study analysis which was 71% of the total participants. At the 6 week point, 7 (17%) had discontinued high fat, low carbohydrate (LCD), 3 (8%) dropped out of the high carbohydrate, low fat (LFD) group and 3 (7%) from the low glycaemic load (LGL) group. The highest drop-out figures were among LCD group with one unable to tolerate the strict carbohydrate restriction, another claiming to be physically unwell and a third individual unable to cope with the financial expense such a diet demanded. Unfortunately funds were not available to provide diet expenses reimbursement. Two subjects discontinued due to unhappiness with the degree of weight loss. The highest retention rate was among the LFD group (79%), but those who discontinued did so out of disappointment with weight loss or dietary allocation as the participants felt that this was the standard and therefore did not perceive that they were receiving any form of intervention (Table 21). In this study retention rates were significantly better than those usually seen in weight loss diet studies. At six months 67%, 82%, and 81% of volunteers (patients) continued in the LCD, LFD, and LGL groups respectively, while at twelve months the figures were 59%, 79% and 76% respectively.

Table 21: Table of Retention and Drop-outs during the Study

	Low Carbohydrate	**Low fat**	**Low Glycaemic Load**	Drop out all groups	Total in Study
Total at baseline	41	38	42		121
Total dropouts by 2 weeks	**6** (1 unable to meet costs of diet)	**2** (1 unhappy with diet allocation)	**3**	11	110
Dropouts by 6 weeks	**1**	**1** (unsatisfied with weight loss)	**0**	13	108
Total dropouts by 3months	4	2 (1 unsatisfied with weight loss)	5 (1 TIA – statin started)	24	97
Dropouts in 3-6months	4 (1 moved to Scotland)	2 (1 developed IHD – statin started)	0	30	91
Dropouts in 6-12months	2 (1 work commitments)	1 (Carcinoma of pancreas)	2	35	86
Total Dropouts	17	8	10	35	86
Total patients in analysis	**24**	**30**	**32**	**35**	86

Figure 13: CONSORT Diagram of Study Recruitment



6.1.1. Baseline Characteristics

The baseline characteristics for subjects participating in the study are shown in (Table 22 - Table 24). A comparison of baseline characteristics of the whole study cohort (n=121) and those completing the entire 12 months (n= 86) demonstrated no difference between the two study cohorts (Table 23). There were no significant baseline demographic differences between the three groups at baseline. The average age was 56 years. Females (n=66, 55%) outnumbered the participating males (n=55, 45 %) at the start of the study and remained so till the end with proportions maintained (n=48, 56% females, n=38, 44% males). The numbers of individuals with diabetes among the groups was small but equally distributed with no statistical difference in the starting HbA1c.

Table 22: Baseline characteristics of all study cohort and completers

	Full Study Cohort	Completed Study
Number of subjects	121	86
Age	56 ± 10.8	57 ± 10.1
Female	66	44
Male	55	42
Diabetes	22	19
Height (m)	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	98.4± 18.7	97.6 ± 17.3
BMI (kg/m^2)	34.4 ± 5.6	34.4 ± 5.4
Waist circumference	108.0 ± 12.1	108.1 ± 11.1
Total cholesterol (mmol/dl)	5.7 ± 1.0	5.7 ± 0.9
HDL-cholesterol (mmol/dl)	1.4 ± 0.4	1.4 ± 0.4
LDL-cholesterol (mmol/dl)	3.4 ± 0.8	3.5 ± 0.8
Total cholesterol/HDL ratio	4.4 ±1.2	4.1 ± 121
Fasting Triglycerides	2.0 ± 1.0	2.0 ± 0.9
Fasting glucose (mmol/dl)	5.9 ± 1.8	5.8 ± 1.6
HbA1c (%)	6.7 ±1.0 (n=22)	6.7 ± 1.0 (n=7)
Systolic BP (mmHg)	140.8 ± 15.5	139.7 ± 15.3
Diastolic BP (mmHg)	85.6 ± 10.2	84.7 ± 9.1
3 Components of MS n(%)	84(69)	63(73)
4 Components of MS n(%)	28(23)	16(19)
5 Components of MS n(%)	9(7)	7(8)
Cardiovascular Risk Score (%)	18.7 ± 11.9	18.9 ± 12.0
Coronary Heart Disease Risk (%)	12.0 ± 8.0	12.2 ± 8.3

Table 23: Baseline Characteristics of Subjects in Study (all subjects recruited)

	Low Carbohydrate	Low fat	Low Glycaemic Load
Number of subjects	41	38	42
Age	51± 12.3	59 ± 10.1	56 ± 8.9
Female	24	21	21
Male	17	17	21
Diabetes	6	7	9
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	102.9± 19.0	93.9 ± 16.5	99.4 ± 19.8
BMI (kg/m^2)	35.3 ± 6.1	33.5 ± 5.2	34.3 ± 5.3
Waist circumference	108.4 ± 11.6	106.5 ± 9.7	109.1 ± 14.0
Total cholesterol (mmol/dl)	5.6 ± 1.0	5.7 ± 1.1	5.8 ± 0.8
HDL-cholesterol (mmol/dl)	1.4 ± 0.5	1.4 ± 0.4	1.3 ± 0.4
LDL-cholesterol (mmol/dl)	3.3 ± 0.8	3.5 ± 1.0	3.4 ± 0.7
Total cholesterol/HDL ratio	4.4 ±1.2	4.5 ± 1.1	4.5 ± 1.2
Fasting Triglycerides	1.9 ± 1.0	2.1± 0.8	2.1 ± 1.1
Fasting glucose (mmol/dl)	5.77 ± 1.74	5.83 ± 1.49	6.18 ± 1.98
HbA1c (%)	6.7 ±1.4 (n=6)	7.0 ± 0.9 (n=7)	6.6 ± 0.8 (n=9)
Systolic BP (mmHg)	139.1± 18.3	141.6 ± 12.7	142.0 ± 14.6
Diastolic BP (mmHg)	84.9 ± 9.5	85.3 ± 10.50	86.5 ± 10.5
3 Components of MS n(%)	31(76)	26(68)	27(664)
4 Components of MS n(%)	7(17)	10(26)	11(26)
5 Components of MS n(%)	3(7)	2(5)	4(10)
Cardiovascular Risk Score (%)	15.0 ± 10.7	20.8 ± 12.0	20.1 ± 11.9
Coronary Heart Disease Risk (%)	9.1 ±6.4	13.5 ± 8.7	13.1 ±8.1

There were no demographic differences between the groups at baseline.

Table 24: Baseline Characteristics of Subjects in Study (subjects who completed and included in per protocol analysis)

	Low Carbohydrate	Low fat	Low Glycaemic Load
Number of subjects	24	30	32
Age	54.5± 11.7	59.6 ± 10.2	56 ± 8.9
Female	15	15	14
Male	9	15	18
Diabetes	4	6	9
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.70 ± 0.10
Weight (kg)	101.5± 19.5	93.4 ± 17.0	99.4 ± 19.8
BMI (kg/m^2)	35.8 ± 6.7	33.4 ± 5.0	34.3 ± 5.3
Waist circumference	109.2 ± 11.7	106.8 ± 10.2	109.1 ± 14.0
Total cholesterol (mmol/dl)	5.6 ± 1.0	5.7 ± 1.1	5.78 ± 0.84
HDL-cholesterol (mmol/dl)	1.4 ± 0.5	1.3 ± 0.4	1.34 ± 0.40
LDL-cholesterol (mmol/dl)	3.4 ± 0.8	3.5 ± 1.0	3.47 ± 0.72
Total cholesterol/HDL ratio	4.3 ±1.1	4.5 ± 1.1	4.52 ± 1.24
Fasting Triglycerides	1.8 ± 1.0	2.1± 0.8	2.11 ± 1.07
Fasting glucose (mmol/dl)	5.7 ± 1.9	5.9 ± 1.6	6.18 ± 1.98
HbA1c (%)	6.5 ±1.4 (n=4)	7.1 ± 1.0 (n=6)	6.57 ± 0.79 (n=9)
Systolic BP (mmHg)	136.5± 17.7	142.6 ± 14.0	142.0 ± 14.6
Diastolic BP (mmHg)	82.3 ± 8.2	86.2 ± 8.8	86.5 ± 10.5
3 Components of MS n(%)	20(83)	20(67)	23(72)
4 Components of MS n(%)	2(8)	8(27)	6(19)
5 Components of MS n(%)	2(8)	2(8)	3(9)
Cardiovascular Risk Score (%)	14.4 ± 9.8	21.9 ± 12.3	20.11 ± 11.9
Coronary Heart Disease Risk (%)	8.9 ±6.0	14.3 ± 8.0	12.8 ±8.6

6.2. Changes in Weight and Waist

Weight and waist measurements were evenly distributed amongst the three groups. Individuals in the LFD group were collectively lighter in weight compared to the other two groups but the difference was not statistically significant (p=0.12 for LFD vs. LCD; p=0.20 for LFD vs. LGL; p=0.78 for LGL vs. LCD in between group ANOVA).

All three groups lost weight with the average overall weight loss being 6.50kg (6.6 %). The highest individual weight loss was 31.5kg (24.3% of total body weight) and the least was a gain of 4.80kg (+4.7 %). Total average weight loss was least in the LFD group, corresponding to suggestions from previous studies that a high carbohydrate intake may encourage weight gain.

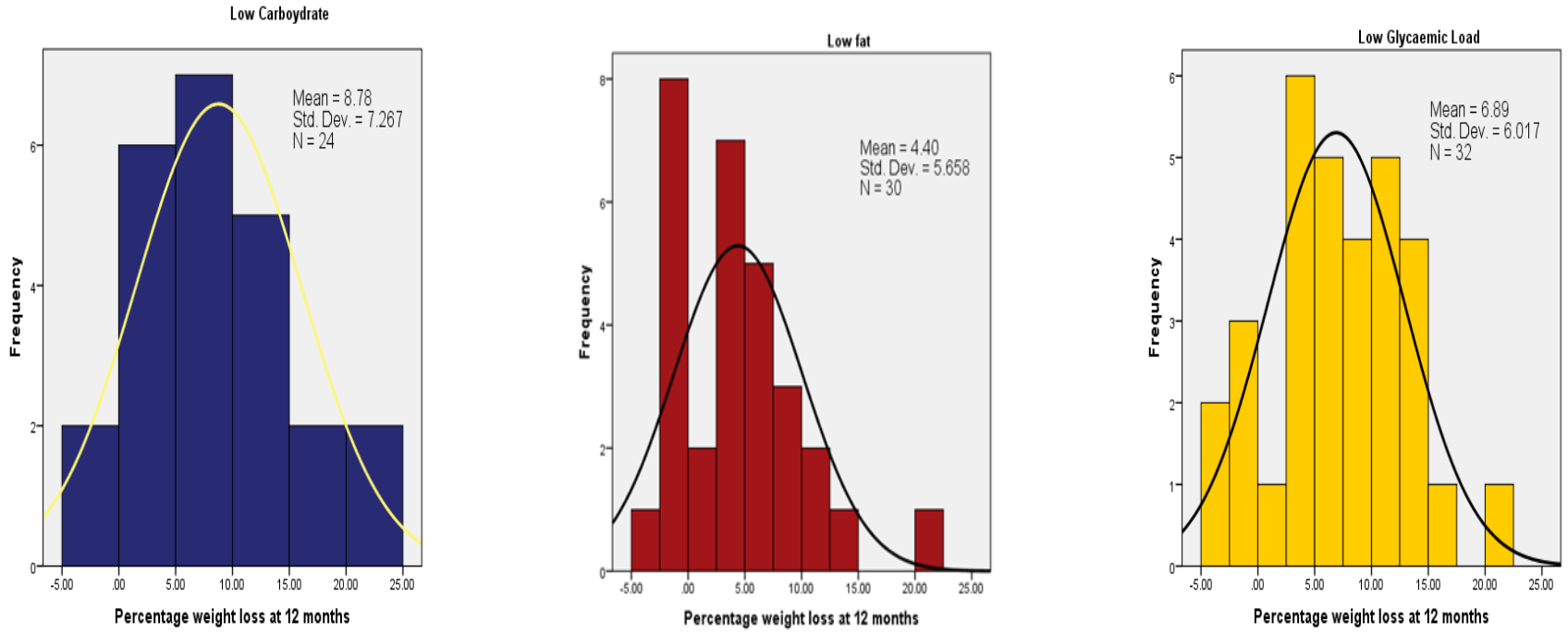
When looking at the twelve best and worst weight loss results, the LCD group appeared more frequently in the best losers' category with six of the subjects, while the LFD group was more prominent in the least losers group (Table 25). The LGL group was evenly distributed among the two categories. Similar results were confirmed on the histograms of percentage weight loss at the end of the study period (Figure 14).

Table 25: The twelve best and worst weight changes among the entire group.

	Best Weight Losers		**Least Weight Losers***
Diet	**Weight loss at 12 month (kg)**	**Diet**	**Weight loss at 12 month (kg)**
Low Carbohydrate	31.5	Low Glycaemic Load	-4.8
Low Carbohydrate	26.9	Low Fat	-4.1
Low Glycaemic Load	25.1	Low Carbohydrate	-3.8
Low Fat	24.1	Low Glycaemic Load	-3.6
Low Carbohydrate	17.4	Low Fat	-1.9
Low Glycaemic Load	15.8	Low Fat	-1.8
Low Carbohydrate	15.6	Low Glycaemic Load	-1.8
Low Glycaemic Load	15.1	Low Fat	-1.7
Low Carbohydrate	14.9	Low Fat	-1.7
Low Fat	14.8	Low Fat	-1.5
Low Carbohydrate	13.2	Low Glycaemic Load	-1.5
Low Glycaemic Load	13.2	Low Carbohydrate	-1.3

*Least weight losers were actually the most weight gainers.

Figure 14: Distribution of percentage weight loss at the end of the study among the three groups



Low Carbohydrate | Low Fat | Low Glycaemic Load

6.2.1. Changes in Weight and Waist as Intention to Treat analysis

All three groups demonstrated reductions in weight, BMI and waist measurements (Table 26). Reductions among the LCD group were greatest (7.8% of total body weight), and least among the LFD group (4.7% of total body weight). Despite these apparent differences there was no statistical difference between the groups in any of the anthropometric measurements at any stage of the study

Table 26: Changes in weight among the three groups (intention to treat)

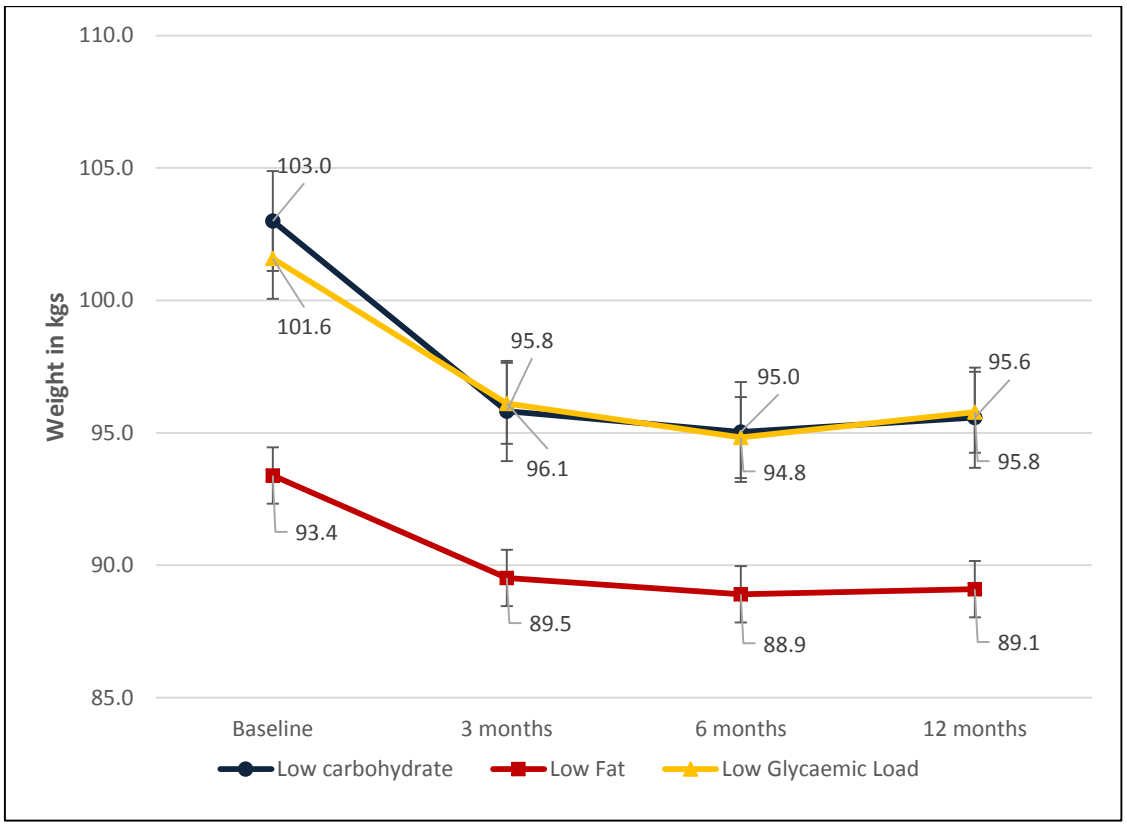


Table 27: Changes in anthropometric measures (intention to treat)

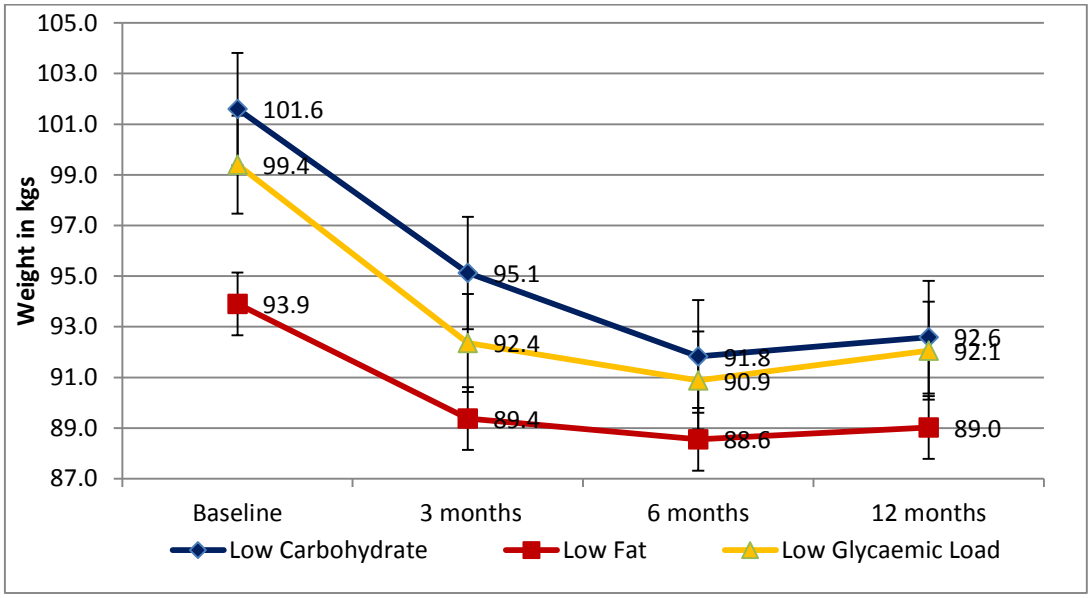
	Randomised Diet								
	Low Carbohydrate			**Low Fat**			**Low Glycaemic Load**		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Weight (kg)									
Baseline	103.0±20.1	152.9	64.6	93.4±16.1	132.0	70.6	101.6±20.43	186.3	71.5
3 months	95.8±19.2♥	148.1	58.3	89.5±15.2♥	129.5	70.1	96.1±21.0♥	186.4	65.7
6 months	95.0±20.0♥	148.0	57.4	88.9±15.4♥	130.8	68.5	94.8±21.4♥	186.4	63.1
12 months	95.6±20.1♥	148.0	57.8	89.1±14.9♥	132.3	68.7	95.8±21.9♥	186.4	61.7
BMI (kg/m2)									
Baseline	35.5±6.4	55.1	26.3	33.4±5.0	47.1	27.2	35.0±5.6	52.7	27.6
3 months	33.0±6.7♥	51.2	24.5	32.0±4.8 ♥	45.7	25.6	33.1±5.4♥	52.7	25.4
6 months	32.7±6.1♥	49.7	25.2	31.8±5.0♥	46.8	25.6	32.6±4.9♥	52.7	24.3
12 months	32.9±6.2♥	49.8	24.4	31.9±4.9♥	45.6	24.6	33.0±6.4♥	52.7	23.8
Waist Circumference (cm)									
Baseline	109.1±12.1	141.0	89.5	106.6±9.6	129.0	88.0	110.5±14.4	167.0	86.0
3 months	102.4±12.3♥	139.0	83.0	101.7±9.7♥	121.5	85.0	105.2±15.0♥	166.0	80.5
6 months	100.5±12.9♥	137.0	76.5	100.0±9.7♥	121.5	81.5	101.9±15.6♥	166.0	77.5
12 months	100.0±13.6♥	137.0	75.5	98.8±9.5♥	122.0	81.5	101.2±16.1♥	166.0	74.0

♥ p <0.001 (p-values are for within group comparisons back to baseline)
Differences between LCD and LFD were significant for weight and BMI at 3 months (p=0.001)
Differences between LCD and LGL were not significant at all stages.
Differences between LFD and LGL were not significant at all stage

6.2.2. Changes in Weight and Waist for Completers

At 3 and 6 months there were significant differences in weight loss between the LCD and LFD groups (p<.0.001). No difference was noted between the LCD and LGL groups or the LFD and LGL group at any point of the study (Figure 15 & Table 28).

Figure 15: Changes in weight among the three groups (completers)



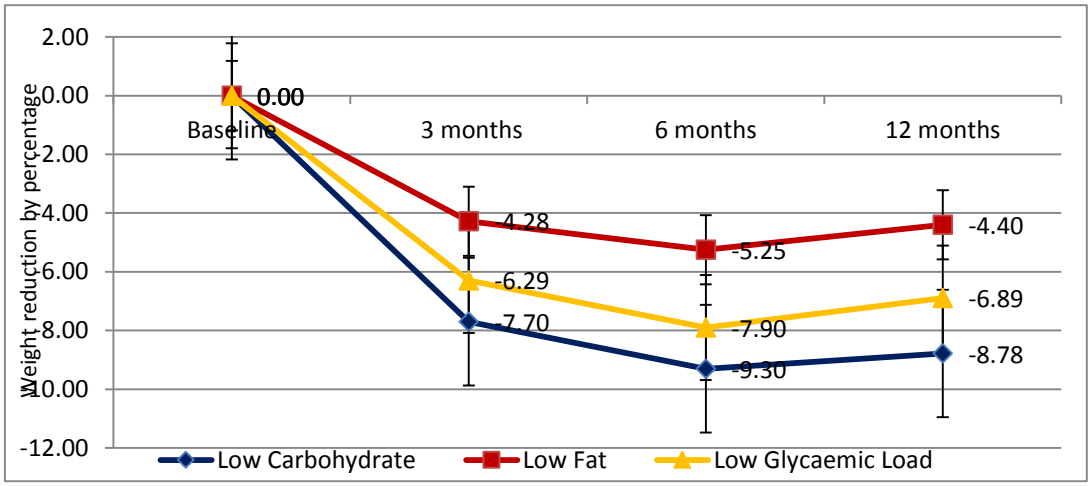
Graph demonstrates the weight of each group at each study interval with standard error

Table 28: Changes in anthropometric measures (completers)

	Randomised Diet								
	Low Carbohydrate			**Low Fat**			**Low Glycaemic Load**		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Weight (kg)									
Baseline	101.5±19.5	146.3	64.6	93.4±17.1	132.00	70.60	98.7±15.3	133.4	71.5
3 months	93.1±18.0♥	136.5	58.3	89.4±15.6♥	129.50	70.10	92.4±14.7♥	118.5	65.7
6 months	91.8±18.9♥	143.7	57.4	88.7±16.1♥	130.80	68.50	90.9±15.3♥	125.1	63.1
12 months	92.6±19.1♥	141.4	57.8	89.0±15.7♥	132.30	68.70	92.1±16.2♥	134.7	61.7
BMI (kg/m2)									
Baseline	35.7±6.7	55.1	26.3	33.4±5.0	47.1	27.2	35.0±5.6	47.8	27.6
3 months	32.8±6.3♥	51.2	24.5	32.0±5.0♥	45.7	25.59	32.1±4.3♥	42.3	25.4
6 months	32.3±6.5♥	49.7	25.2	31.8±5.0♥	46.8	25.6	31.6±4.7♥	44.9	24.3
12 months	32.6±6.6♥	49.8	24.4	31.9±4.9♥	45.6	24.6	32.1±5.3♥	48.3	23.8
Waist Circumference (cm)									
Baseline	109.2±11.7	135.0	89.5	106.8±9.6	129.0	88.0	110.5±14.4	136.0	86.0
3 months	101.3±11.4♥	123.0	83.0	101.8±9.8♥	121.5	85.0	101.5±11.2♥	124.0	80.5
6 months	98.6±12.4♥	126.0	76.5	99.8±9.7♥	121.5	81.5	98.9±11.5♥	125.5	77.5
12 months	97.8±13.3♥	123.5	75.5	98.4±10.0♥	122.0	81.5	97.8±12.0♥	128.0	74.0

♥ p <0.001 (p-values are for within group comparisons back to baseline)
Differences between LCD and LFD were significant for weight and BMI at 3 & 6 months (p<0.001) and waist (p<0.001) at 3 & 6 months.
Differences between LCD and LGL were not significant at all stages.
Differences between LFD and LGL were not significant at all stages.

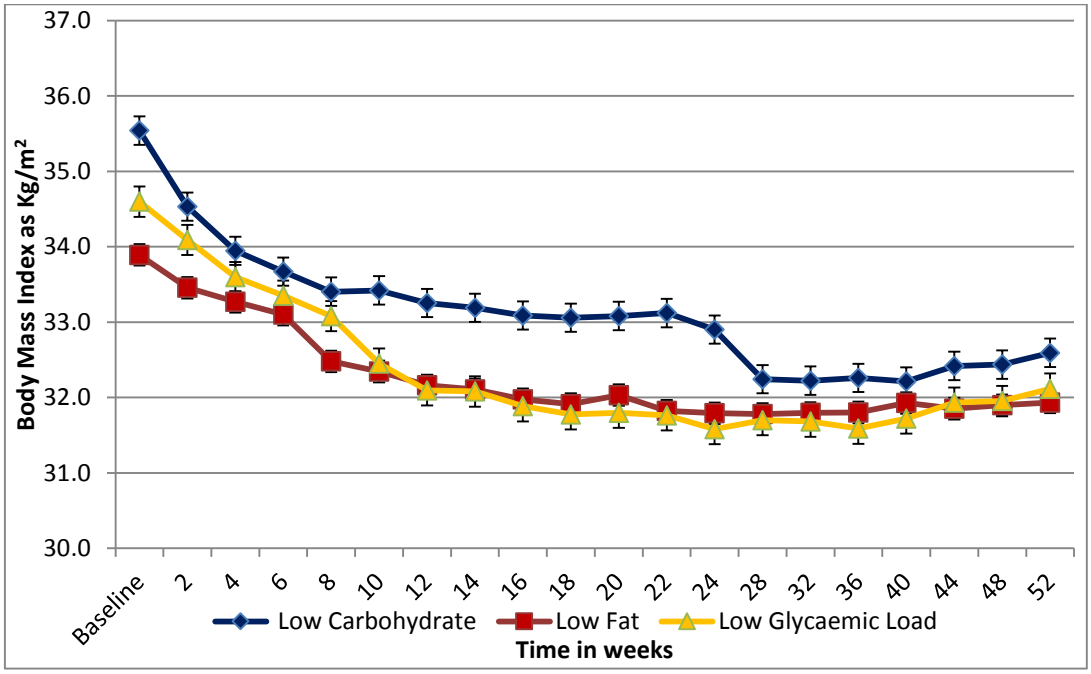
Figure 16: percentage weight reductions for the three groups



Graph demonstrates the percentage weight loss of each group at each study interval with standard error

Changes in BMI (Table 28 & Figure 17) followed a similar pattern as weight with measurements falling maximally at 6 months, 33.3, 31.8 and 31.6kg/m^2 for LCD, LFD and LGL respectively, rising somewhat at 12 months (32.6, 31.9 and 32.1kg/m^2 respectively). Between group changes were significant between LCD and LFD at all study intervals ($p<0.05$), and LCD and LGL at 3 months only.

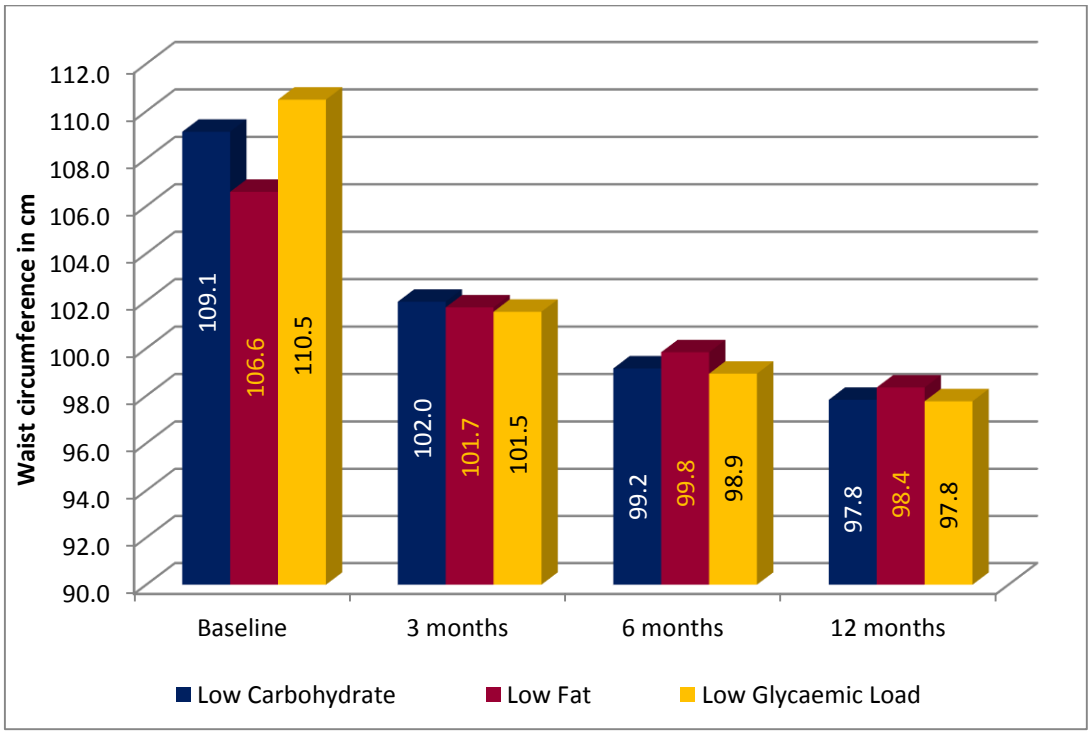
Figure 17: Variations in Body Mass Index throughout the study period



Graph demonstrates the BMI of each group with standard error

Reductions in waist measurement (Figure 18 & Table 28) occurred within all three groups and persisted at all stages of the study interval despite weight measurements beginning to rise at 6 months. The combined mean waist measurement for the groups at the start was 108cm and 98cm at finish, an average loss of 8.7%. Although no statistical difference existed between the groups at all study intervals the percentage of waist circumference reduction was significant for LCD (6.7 % 9.1%) vs. LFD 4.5 % 6.2%) at 3 and 6 months ($p<0.01$) and for LCD (6.7%) vs. LGL (6.1%) at 3 months ($p<0.05$).

Figure 18: Changes in waist circumference measurements



P<0.01 when comparing each study interval to baseline for all three intervention groups.

6.3. Changes in Components of the Metabolic Syndrome

All subjects taking part in the study fulfilled the NCEP-ATP criteria for metabolic syndrome at the start of the study (i.e. displayed at least three of the five criterion which define the syndrome) (Table 1, Figure 19). The exception was those individuals who were already on medication for diabetes or hypertension as baseline bloods were potentially normal due to the effect of medication. Analysis of the number of individuals with metabolic syndrome at start and end of study included those individuals who were already on anti-hypertensive medication regardless of baseline blood pressure readings (**Table 31-Table 33**).

Most individuals displayed 3 to 4 criteria of the metabolic syndrome (average =3.3) at the start of the study with equal distribution among the three groups, (average for LCD= 3.3, LFD= 3.4, LGL= 3.4) at the start (Table 31).

Reductions in the number of individuals fulfilling the criteria for metabolic syndrome were noted across the three groups with the greatest of these reductions noted among the LCD group where there was an 83% reduction in the number of individuals who had 3 or more components to fulfil the diagnostic criteria for MS. The fall among the other two groups was 50% for LFD and 62% for LGL.

Overall in combination, approximately 60% of the completers no longer had the features that would make them eligible for the diagnosis of metabolic syndrome by the end of the study (**Table 34**).

Whereas at the start of the study only two individuals had controlled blood pressure on antihypertensive therapy, the numbers increased to 18 at the end of the study with only 62 individuals classified as hypertensive (i.e. systolic blood pressure >130mmmHg). Reductions and changes in blood pressure medication are discussed in the following section.

Table 29: Prevalence of 3 or more abnormalities of Metabolic Syndrome at baseline for whole study cohort

Components of Metabolic Syndrome	Low Carbohydrate N=41			Low Fat N= 38			Low Glycaemic Load N= 42			Combined N= 121		
	M	**F**	**All**	**M**	**F**	**All**	**M**	**F**	**All**	**M**	**F**	**All**
3 Components	10	21	31	9	17	26	13	14	27	32	52	84
4 Components	5	2	7	6	4	10	6	5	11	17	11	28
5 Components	2	1	3	2	0	2	2	2	4	6	3	9

Table 30: Prevalence of 3 or more abnormalities of Metabolic Syndrome at baseline for the study completers by percentage

Components of Metabolic Syndrome	Low Carbohydrate N=24 (%)			Low Fat N= 30 (%)			Low Glycaemic Load N= 32 (%)			Combined N= 86 (%)		
	M	**F**	**All**	**M**	**F**	**All**	**M**	**F**	**All**	**M**	**F**	**All**
3 Components	67	93	83	47	87	67	64	78	72	58	85	73
4 Components	22	0	8	40	13	27	29	11	19	32	8	19
5 Components	11	7	8	13	0	7	7	11	9	10	6	8

Figure 19: Prevalence of Metabolic Syndrome at Baseline among the Completers

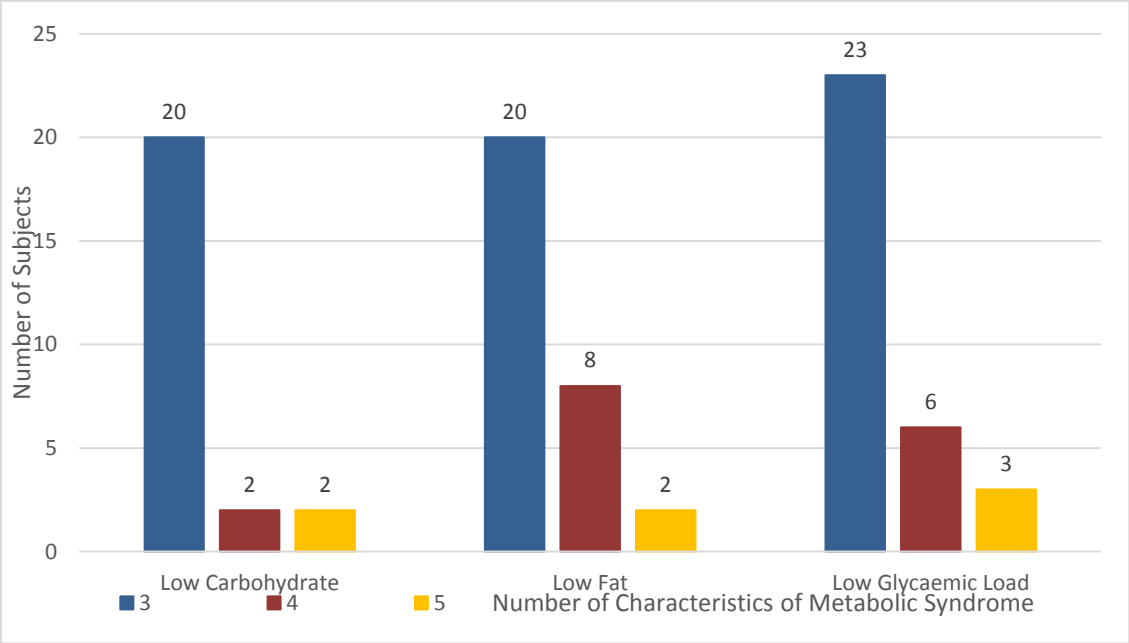


Figure 20: Prevalence of Metabolic Syndrome at End of Study among the Completers

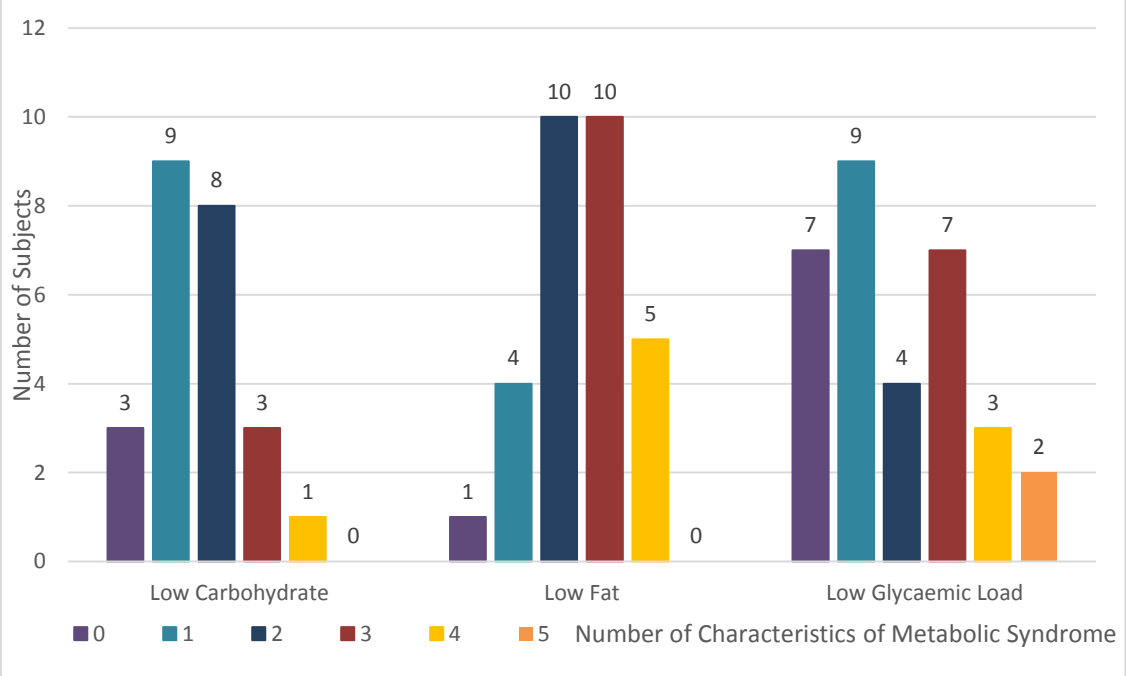


Table 31: Number of Individuals fulfilling each of the criteria for Metabolic Syndrome at the start (the whole cohort)

	Low Carbohydrate N=41			Low Fat N= 38			Low Glycaemic Load N= 42			Combined N= 121		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL	M	F	**TOTAL**
Waist Measurement	15	22	37	14	21	35	21	17	38	50	66	**116**
Fasting Glucose (Diabetes)	7 (1)	5 (1)	12 (2)	7	3	10	8 (1)	9 (1)	17 (2)	22 (2)	17 (2)	**39 (4)**
Triglycerides	11	7	18	14	8	22	17	9	26	42	24	**66**
HDL-cholesterol	6	7	11	5	5	10	7	4	11	18	16	**34**
Systolic Blood pressure	17	21	38	17(1)	20(1)	37(2)	21	21	42	55	62	**117(2)**
Diastolic Blood pressure	11	14	25	13	14	27	18	11	29	42	39	**81**

Figures in parentheses () indicate number of individuals with diabetes or on anti-hypertensive therapy with normal blood pressure reading.

Table 32: Number of Individuals fulfilling each of the criteria for Metabolic Syndrome at the start (completers)

	Low Carbohydrate N= 24			Low Fat N= 30			Low Glycaemic Load N= 32			Combined N= 86		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL	M	F	**TOTAL**
Waist Measurement	8	14	22	13	15	28	12	17	29	33	46	**79**
Fasting Glucose (Diabetes)	4 (3)	4 (1)	8 (4)	7	2	9	6 (1)	7 (1)	13 (2)	17 (4)	13 (3)	**30**
Triglycerides	6	5	11	13	6	19	10	7	17	29	18	**47**
HDL-cholesterol	3	4	7	5	5	10	4	3	7	12	12	**24**
Systolic Blood pressure	9	12	21	15 (1)	15 (1)	30 (2)	14	18	32	38	45	**83(2)**
Diastolic Blood pressure	7	10	17	12	10	22	12	8	20	31	28	**59**

Figures in parentheses () indicate number of individuals with diabetes or on anti-hypertensive therapy with normal blood pressure reading.

Table 33: Number of Individuals fulfilling each of the criteria for Metabolic syndrome at the end (completers)

	Low Carbohydrate N= 24			Low Fat N= 30			Low Glycaemic Load N= 32			Combined N= 86		
	M	F	TOTAL	M	F	TOTAL	M	F	TOTAL	M	F	**TOTAL**
Waist Measurement	6	10	16	8	11	19	6	11	17	20	32	**52**
Fasting Glucose (Diabetes)	3	2	5	7 (1)	2	9	5 (2)	5 (2)	10 (4)	15 (3)	9 (2)	**24 (5)**
Triglycerides	2	4	6	5	4	9	7	6	13	14	14	**28**
HDL-cholesterol	1	0	1	3	4	7	1	3	4	5	7	**12**
Systolic Blood pressure	6 (2)	9 (2)	15 (4)	15 (5)	12 (3)	27 (8)	11 (3)	9 (3)	20 (6)	32 (10)	30 (8)	**62 (18)**
Diastolic Blood pressure	1	2	3	6	4	10	5	4	9	12	10	**22**

Figures in parentheses () indicate number of individuals with diabetes or on anti-hypertensive therapy with normal blood pressure reading.

Table 34: Percentage Reductions in each Parameter of the Metabolic Syndrome

	Low Carbohydrate N= 24			Low Fat N= 30			Low Glycaemic Load N= 32			Combined N= 86		
	M	F	**TOTAL (%)**	M	F	**TOTAL (%)**	M	F	**TOTAL (%)**	M	F	**TOTAL (%)**
Waist Measurement	25	29	**27**	39	27	**32**	50	35	**41**	39	30	**34**
Fasting Glucose (Diabetes)	25	50	**38**	0	0	**0**	17	29	**23**	12	31	**20**
Triglycerides	67	20	**46**	62	33	**53**	30	14	**24**	52	22	**40**
HDL-cholesterol	67	100	**86**	40	20	**30**	75	0	**43**	58	42	**50**
Systolic Blood pressure	33	25	**29**	0	20	**10**	21	50	**38**	16	33	**26**
Diastolic Blood pressure	86	80	**82**	50	60	**55**	58	50	**55**	62	64	**63**
TOTAL REDUCTION			**51**			**30**			**37**			**39**

6.4. Changes in Blood Pressure

Changes in blood pressure readings were generally small (Table 35). Reductions in systolic blood pressure were 2.7, 4.8, & 5.7% in the LCD group at 3, 6 and 12 months ($p<0.01$ at 6 & 12 months), 2.3, 2.3 & 1.7% for LFD (p=NS), and 1.6, 5.0 & 5.5% for LGL ($p<0.01$ at 6 and 12 months) No differences were demonstrated in between group analysis.

Reductions in diastolic blood pressure were +1.3, -3.0 & -4.0% in the LCD group at 3, 6 and 12 months respectively ($p<0.05$ at 12 months), 1.2, 2.7 and 3.9% in LFD ($p<0.05$ at 12 months), and 1.6, 5 and 5.5% in the LGL group ($p<0.01$ at 6 & 12 months) for the same time intervals. (**Figure 21** & **Table 36**).

Changes in blood pressure did not correlate to changes in weight in the whole cohort nor for LCD or LFD. Diastolic blood pressure changes in the LGL group positively correlated to weight at 3, 6 & 12 months (r =0.41, 0.43, & 0.47, $p<0.05$ for all).

Table 35: Blood pressure at each of the study intervals

	Low Carbohydrate	Low Fat	Low Glycaemic Load
Systolic BP (mmHg)			
Baseline	138.5±17.9	141.7±13.4	141.2±15.2
3 months	133.7±17.3	138.21±15.5	137.2±14.9
6 months	129.8♥±14.6	138.5±18.1	132.8♦±17.7
12 months	127.8♦±14.2	140.1±18.3	130.7*±13.2
Diastolic BP (mmHg)			
Baseline	84.6±9.7	85.20±10.9	86.41±10.8
3 months	84.8±8.7	84.42±9.5	84.44±7.9
6 months	79.7±7.6	82.77±9.3	80.38♥±7.9
12 months	78.7♦±7.4	82.60♦±8.9	80.41*±7.1

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values are for within group comparisons back to baseline)

Table 36: Mean blood pressure changes across the groups

	Low Carbohydrate	Low Fat	Low Glycaemic Load
Systolic BP (mmHg)	**Mean**	**Mean**	**Mean**
3 months	-4.6±15.3	-3.8±14.6	-3.3±16.9
6 months	-7.2±12.8*	-3.6±14.9	-7.6±17.4*
12 months	-8.8±15.8*	-6.5±25.2	-8.6±16.3*
Diastolic BP (mmHg)			
3 months	+0.7±7.03	-0.1±11.1	-1.7±8.6
6 months	-2.8±7.7	-2.5±8.7	-5.8±8.5*
12 months	-.3.6±7.1♦	-5.3±11.9♦	-4.7±8.4*

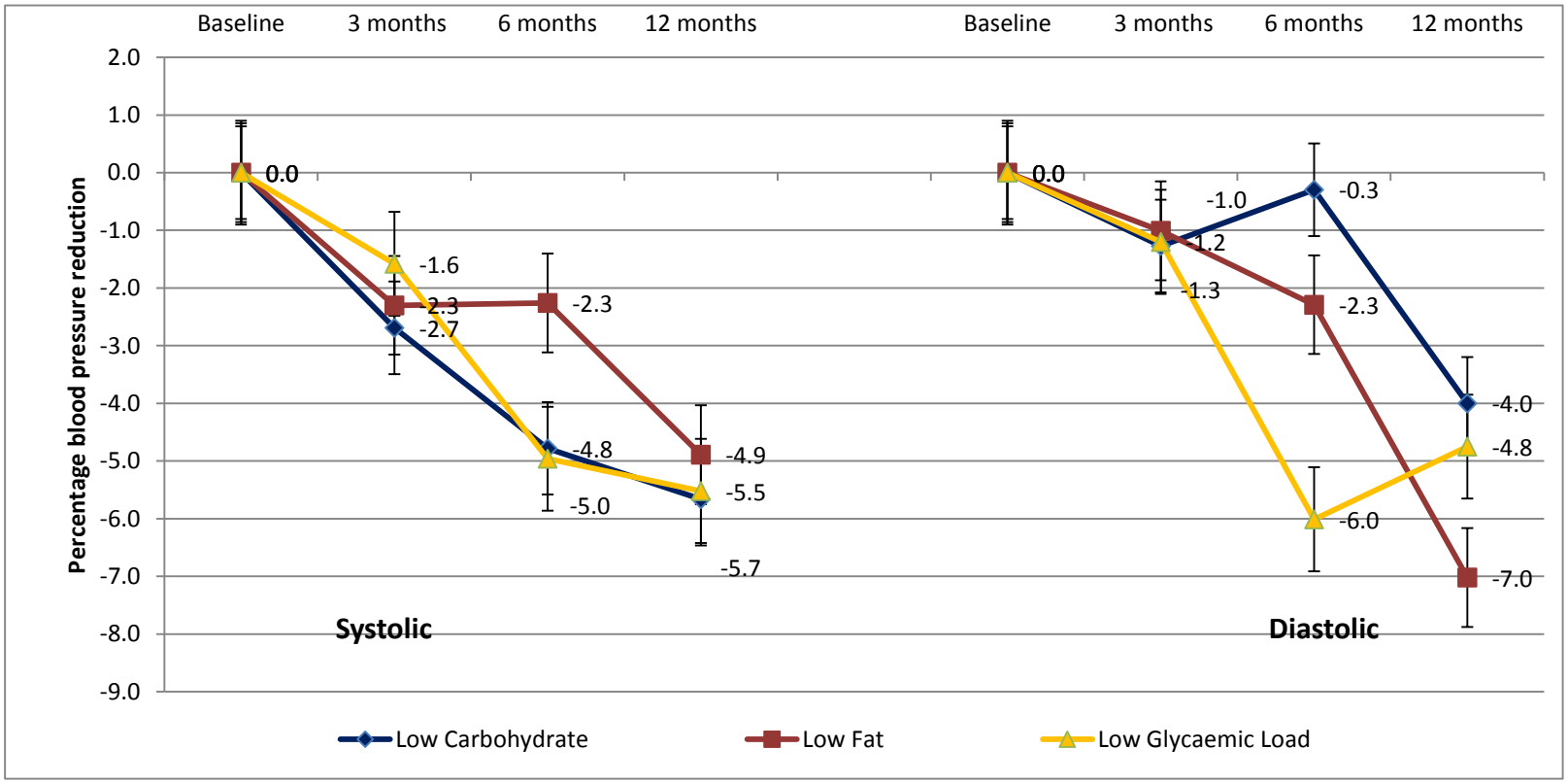
♦ p< 0.05, * p <0.01 (p-values are for within group comparisons back to baseline)

As individuals who were known to be hypertensive had not been excluded from the study the number of antihypertensive agents individuals were taking were documented. Sixty-two patients were taking some form of antihypertensive agents at the start of the study and only 53 at the end. The average number of agents used at the start of study was 2.0 per individual, and 2.1 at the end. Thirteen people discontinued all antihypertensive medication, three started new antihypertensive therapy, six had dose reductions and seven had dose increases. Table 37 outlines those who had alterations in blood pressure medication throughout the study.

Table 37: Changes in the number of antihypertensive agents used

	Low Carbohydrate		Low Fat		Low Glycaemic Load		Total	
	Start	End	Start	End	Start	End	Start	End
On 5 agents	0	1	2	1	0	0	2	2
On 4 agents	2	0	2	3	0	0	4	3
On 3 agents	3	1	3	7	2	2	8	10
On 2 agents	5	6	13	9	9	7	27	22
On 1 agent	8	5	7	6	6	5	21	16
On no agents	1	6	1	2	1	4	3	12
Total on antihypertensive	18	13	27	26	17	14	62	53

Figure 21: Percentage reductions in systolic and diastolic blood pressure readings



Graph demonstrates the changes in blood pressure within each group at each study interval with standard error

Changes in Lipid Profile

The changes in total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides for the three groups are tabulated in Table 38 & 39 and Figure 22.

Total cholesterol levels remained stable in the LFD group (5.76, 5.75, 5.63, 5.75mmol/l) at 0, 3, 6 & 12 months respectively In the LCD group there was a non-significant trend to higher total cholesterol levels (5.51, 5.56, 5.85, 5.77mmol/l), while in the LGL group (5.77, 5.46, 5.56, 5.59mmol/l) the trend appeared to be downward ($p<0.01$ at 3 months). The percentage changes in total cholesterol between LCD (+2.0 & +5.6%) and LGL (-6.4 and -4.6% -) were significant at 3 and 6 months ($p<0.05$ for both).

Changes in LDL-cholesterol followed a similar pattern to total cholesterol. LDL-cholesterol in LFD remained stable (2.6, -0.6, & 2.4%) at 3, 6 & 12 months; (p=NS) In the LCD group, LDL-cholesterol trended up (3.8, 8.3 & 5.7% (p=NS), and On the LGL diet, LDL-cholesterol fell at 3 months ($p<0.05$), significance not being maintained at 6 and 12 months (-5.3, -2.5 & -4.8%). Between group changes were statistically relevant at three months for LGL vs. LCD ($p<0.05$).

HDL-cholesterol rose in all three groups with levels rising the most 17.5, 17.7 & 18.6%; (all $p<0.01$) in the LCD group at 3, 6 and 12 months, the least 0.6, 5.1 & 8%; (p=NS) in the LFD group and moderately 2.0, 7.0% & 14.7%; ($p <0.05$ at 6 & 12 months) in the LGL group for the same time intervals. These changes were statistically significant between the groups at 3 and 6 months for LCD vs. LFD (both $p<0.01$), and LCD vs. LGL (both $p<0.05$). LGL vs. LFD was significant at 3 months ($p<0.01$).

Changes seen in Non HDL-cholesterol were similar to that noted for total and LDL-cholesterol. Non HDL-cholesterol fell by 8.9, 7.7 and 8.6% in the LGL group, 0.1, 3.3, and 0.4% for LFD at 3, 6 and 12 months. In the LCD group levels fell at 3 months (-2.7%), rose at 6 months (+2.2%) and returned to baseline at the end (-0.2%). The changes were not significant within or between the groups at any stage.

With the rise in HDL-cholesterol levels total cholesterol to HDL-cholesterol ratios fell in all three interventions by 11.8, 4.7, 9.7% at 3, 6 and 12 months, ($p<0.01$ at 6 & 12 months) in LCD, 5.7, 6.5 and 9.1% (all $p<0.01$) in LGL, and +0.1, 5.2, and 5.9% in the LFD group ($p<0.05$ at 12 months) at the same time intervals. Differences in the change in total cholesterol to HDL-cholesterol ratios between groups was significant at 3 months for LCD vs. LFD ($p<0.01$).

A reduction in triglyceride levels was noted among all three groups. Greatest reductions were within the first six months, the period considered to be the active phase of the dietary interventions. Reductions were 22.6, 10.2 and 14.3% (all $p<0.05$) for LCD at 3, 6 and 12 months, 11.7, 14.1, and 8.4% ($p<0.05$ at 3 & 6 months) for LFD, and 15.9, 23.2 and 11.8% (all $p<0.01$) for LGL. At no stage in the study were between group differences noted.

HDL-cholesterol levels negatively correlated with weight (r= -0.24, -0.23, -0.26 & -0.25; all $p<0.05$) at 0, 3, 6 and 12 months when the three groups were analysed as a cohort. These changes were not noted when the groups were analysed individually. None of the other lipid parameters demonstrated any form of correlation to weight, despite the reduction in triglycerides.

Table 38: Changes in lipid profile over the 12 month study period

	Dietary Intervention								
	Low Carbohydrate			Low Fat			Low Glycaemic Load		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Total cholesterol (mmol/dl)									
Baseline	5.51±1.09	8.6	3.6	5.76±1.10	8.2	5.8	5.77±0.84	7.7	4.1
3 months	5.56±0.94	7.7	4.1	5.75±1.35	9.1	3.0	5.46±0.82*	7.6	3.5
6 months	5.85±0.99	7.7	4.2	5.63±1.27	8.2	2.4	5.56±0.97	7.8	3.3
12 months	5.77±1.01	7.5	3.7	5.75±1.20	7.8	2.8	5.59±0.82	7.2	4
LDL-cholesterol (mmol/l)									
Baseline	3.24±0.78	4.9	1.9	3.51±0.99	5.8	1.0	3.59±0.71	5.0	2.1
3 months	3.38±0.92	5.8	1.8	3.62±1.21	6.5	1.0	3.33±0.77♦	5.3	1.3
6 months	3.59±0.98	5.3	1.9	3.50±1.07	5.8	0.8	3.45±0.92	5.8	1.0
12 months	3.47±0.95	5.4	1.9	3.52±0.99	5.6	1.0	3.33±0.79	4.9	1.6
HDL-cholesterol (mmol/l)									
Baseline	1.34±0.43	3.2	0.7	1.36±0.35	2.1	0.8	1.35±0.42	2.4	0.8
3 months	1.59±0.47♥	0.8	3.4	1.34±0.34	2.1	0.8	1.42±0.44	2.5	0.9
6 months	1.63±0.57*	0.8	3.4	1.41±0.40	2.2	0.8	1.48±0.40*	2.6	0.8
12 months	1.66±0.66*	1.0	4.0	1.44±0.39♦	2.2	0.9	1.58±0.50*	2.8	0.9
Non HDL-cholesterol (mmol/l)									
Baseline	4.18±1.09	7.5	2.6	4.40±1.03	6.8	2.2	4.42±0.84	6.7	2.9
3 months	3.97±1.00	6.3	2.1	4.41±1.27	7.5	2.0	4.04±0.91	6.4	1.7
6 months	4.22±1.17	6.4	2.1	4.22±1.15	6.8	1.6	4.09±1.02	6.5	1.4
12 months	4.11±1.09	6.1	2.2	4.31±1.07	6.4	1.9	4.02±0.94	6.1	2.2

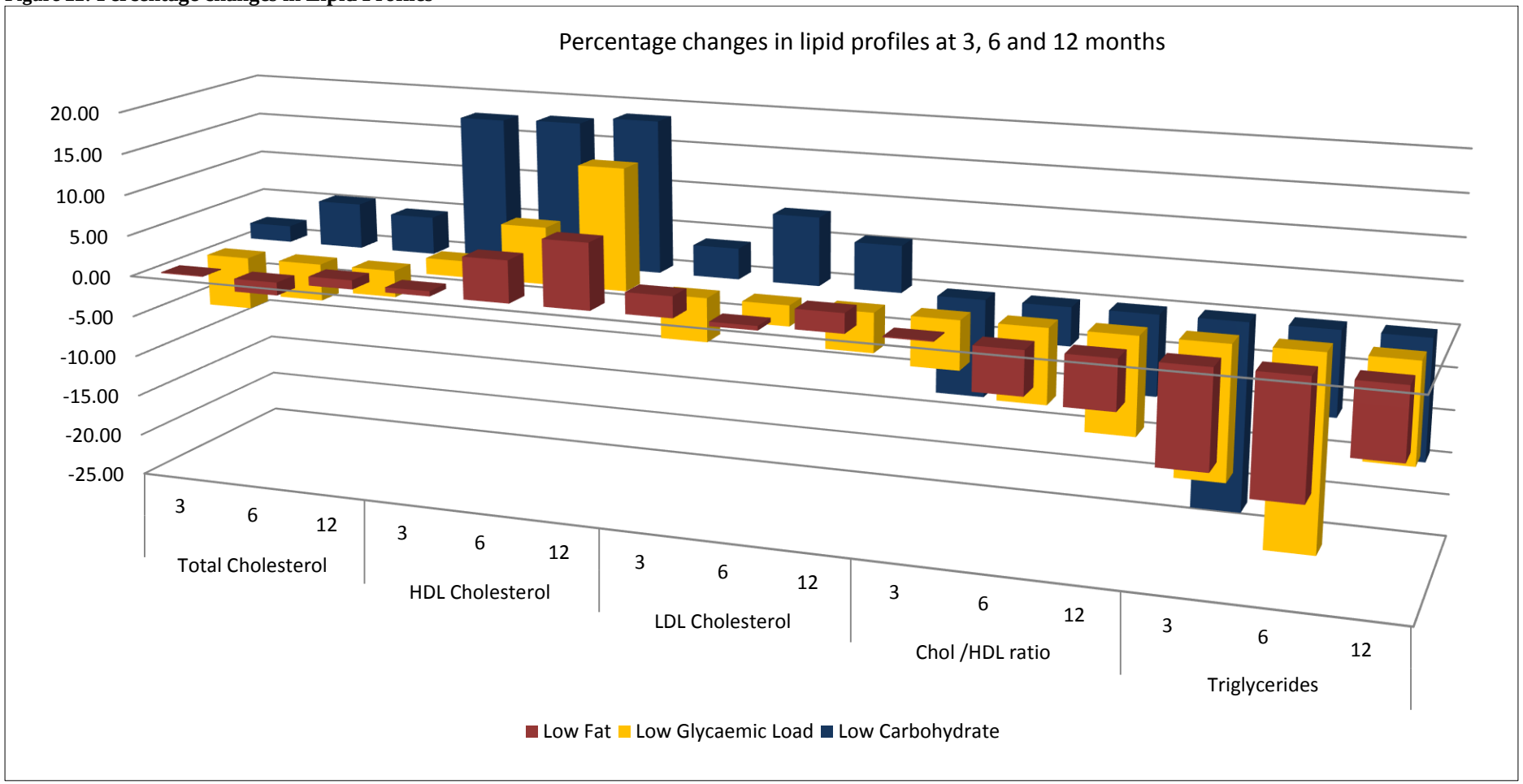
Table 39: Changes in lipid profiles cont...

	Dietary Intervention								
	Low Carbohydrate			Low Fat			Low Glycaemic Load		
Triglycerides (mmol/l)									
Baseline	1.9±0.9	4.8	0.8	2.1±0.8	4.0	1.0	2.0±0.9	4.0	0.6
3 months	1.3±0.5*	2.7	0.6	1.8±0.7*	3.9	0.5	1.6±0.8♥	3.8	0.5
6 months	1.4±0.7♦	2.8	0.6	1.7±0.83♦	4.7	0.5	1.4±0.7♥	3.1	0.5
12 months	1.4±0.7♦	2.9	0.5	1.76±0.83	4.0	0.5	1.5±0.8*	3.9	0.5
Cholesterol/HDL-chol. Ratio									
Baseline	4.4±1.20	7.8	1.9	4.5±1.1	6.9	2.7	4.5±1.2	7.7	2.5
3 months	3.7±1.0♥	6.3	1.6	4.5±1.3	7.9	2.6	4.2±1.3*	7.2	1.9
6 months	3.9±1.5	9.0	1.6	4.2±1.1♦	6.3	2.3	4.0±1.2♥	6.8	1.7
12 months	3.8±1.1*	6.1	1.6	4.2±1.0♦	6.8	2.7	3.9±1.2*	6.5	1.9

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values are for within group comparisons back to baseline)

Differences in changes in total cholesterol between LCD & LGL were significant at 3 & 6 months ($p<0.05$)
Differences in changes in HDL- cholesterol between LCD and LGL were significant at 3 months and between LCD and LFD at 3 & 6 months ($p<0.01$)
Differences in changes in LDL-cholesterol between LGL and LFD were significant at 3 months ($p<0.05$)

Figure 22: Percentage changes in Lipid Profiles



6.5. Changes in Cardiovascular risk

Reductions in CVD risk (Table 40) were 16.9, 15.6, and 15.8% (all $p<0.01$) for LCD, 6.6, 8.9, and 3.5% ($p<0.05$ at 3months) for LFD and 3.9, 18 and 15.4% (all $p<0.05$)) for LGL at 3, 6 and 12 months (Figure 24) Between group differences were significant for the LCD vs. LFD at 3, 6 and 12 months (all $p<0.01$) but not LFD vs. LGL or LCD vs. LGL.

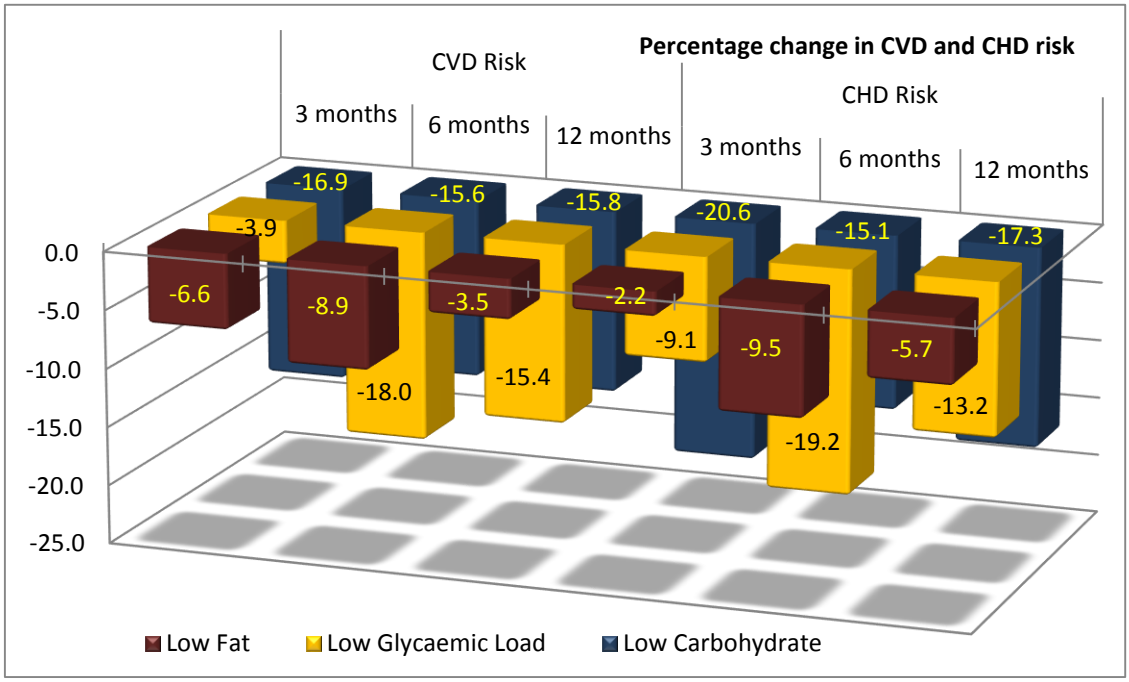
CHD risk reductions were similar in pattern to the CVD reductions. Within group changes in LCD and LGL were statistically significant at all study intervals and only at 6 months for LFD ($p<0.05$). Between group differences were significant for the LCD vs. LFD at 3, 6 and 12 months (all $p<0.01$) but not LFD vs. LGL or LCD vs. LGL.

Table 40: CVD and CHD 10 year risk calculations at each study interval

	Low Carbohydrate	Low Fat	Low Glycaemic Load
	Mean ± SD	Mean ± SD	Mean ± SD
Cardiovascular risk (%)			
Baseline	15 ±1	21 ± 1	20 ± 1
3 months	12 ± 9*	22 ± 11	18 ± 11♦
6 months	12 ± 9♦	20 ± 12	15 ± 11*
12 months	12 ± 8*	21 ±12	16 ± 12*
Coronary Heart Disease risk (%)			
Baseline	9 ± 6	14 ± 9	13 ± 8
3 months	7 ± 5*	13 ± 7	11 ± 7*
6 months	7 ± 6	12 ± 8♦	10 ± 7*
12 months	7 ± 5*	13 ± 8	11 ± 8♦

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values are for within group comparisons back to baseline)

Figure 23: Percentage improvements in CVD and CHD risk



7. CHAPTER 7: Results from Dietary Analysis

7.1. Introduction & methodology

When starting this study it was the intention to perform a comprehensive dietary analysis looking at the subjects' weighed food and oral intake prior to entering the study and then at various intervals throughout the study. The intention was to look at the participants' dietary changes, and concordance with the prescribed dietary regimen. Participants were supplied with weighing scales (Salter aquatronic electronic kitchen scales, Salter Housewares HoMedics House, Tonbridge, Kent) and asked to complete weighed food diaries at various intervals and for the following lengths of time –

1.	Prior to starting study	7 day food diary
2.	6 weeks	4 day food diary
3.	3 months	3 day food diary
4.	6 months	3 day food diary
5.	End of study	3 day food diary

Data from the completed food diaries were entered into a dietary analysis programme. The programme used was DietPlan6 version 6.15 supplied by **Forestfield** Software Ltd, Horsham, United Kingdom.

The data from the consequent analysis were downloaded onto an Excel spreadsheet and contained such details as caloric intake, and a breakdown of carbohydrate, protein, fat and its subgroups intake as well as some of the minerals.

Sixty subjects were randomly chosen by an independent party for dietary analysis. Of the chosen food diary sets, 7 were excluded as they were considered to be substantially incomplete leaving 53 to be included in the analysis (Table 41).

7.2. Results

There was a slightly higher number of female diaries in total which was in proportion to the ratio of males to female recruited to the study (Table 41). Analysis for the LCD and LGL groups contained almost double the number of females as men while the reverse is true in the LFD group. The disparity could be accounted for within the LCD group which did have a larger proportion of females from the start.

Table 41: Number of food diaries analyzed at each study interval

Time Interval	Low Carbohydrate	Low Fat	Low Glycaemic Load	Total
Male : Female	6:11	11:6	7:11	24:28
Pre study	17	17	18	52
6 weeks	17	16	17	50
3 months	17	16	17	50
6 months	16	14	18	48
End of study	13	17	16	46

7.3. Compliance to diet

The food diaries were collected to assess concordance with the diet and whether individuals were meeting the recommended targets. The LCD group just about achieved the minimum expected level of protein intake, kept to target on fat intake and overshot the carbohydrate intake by the end of the study (Table 42). The LFD group increased protein intake by up to 25% throughout the study. Interestingly protein intake in this group rose rather than decreased with randomisation despite the restrictions. Their carbohydrate intake was equal to what would be expected at that time period. They were within their fat recommendations managing to reduce saturated fat to comply. The LGL group were compliant with protein and carbohydrate intake and most of the fat intake apart from saturated fat which was 20 -30% higher than recommended. Of note the changes made to the diets were enough to make the groups different for all the parameters at 6 weeks ($p<0.01$).

Table 42: Confirmation of compliance to diet

	Low Carbohydrate		Low Fat		Low Glycaemic Load	
	Target	Achieved	Target	Achieve	Target	Achieve
Protein	%	%	%	%	%	%
Baseline	30-40	20	10-15	18	25	19
6 weeks		30		19		25
3 months		30		19		25
6 months		28		20		24
12 months		28		22		23
Carbohydrate	gms	gms	%	%	%	%
Baseline	10-12	220	50-60	41	40	44
6 weeks	40	40		47		34
3 months	40-80	42		47		36
6 months	40-100	62		47		37
12 months	40-100	114		43		38
Fat	%	%	%	%	%	%
Baseline	50-60	33	<30	32	35	33
6 weeks		57		28		38
3 months		56		26		34
6 months		52		27		35
12 months		44		29		35
Saturated Fat (gms)	%	%	%	%	%	%
Baseline	NS	12	<10	12	<10	12
6 weeks		22		9		13
3 months		22		9		12
6 months		20		9		11
12 months		16		10		12
Monounsaturated Fats	%	%	%	%	%	%
Baseline	NS	12	NS	12	<15	11
6 weeks		2		10		14
3 months		21		9		13
6 months		18		10		13
12 months		16		10		13
Polyunsaturated Fats (gms)	%	%	%	%	%	%
Baseline	NS	6	<20	5	<10	6
6 weeks		8		6		7
3 months		8		5		6
6 months		8		5		7
12 months		7		6		7

Measurements reported as percentage of daily calorie intake. NS=Not specified

7.4. Caloric Intake

At start the average calorie intake was approximately 2000Kcal per day across the study population. Reductions in caloric intake was seen across the three groups with reductions of 21.6, 19.2, 18.2, and 12.2% ($P< 0.01$ at 6 weeks, 3 and 6 months) in LCD, 23.5, 24.9, 21.5, & 21.7% ($p< 0.05$ at 6 weeks, 3, 6 & 12 months) in LFD, and 9.6, 16.4, 16.5, & 7.2% ($p< 0.05$ at 6 weeks, 3 and 6 months) in LGL at 6 weeks, 3, 6 and 12 months respectively (Table 43, Figure 24). Changes in caloric intake were not statistically different between thee three groups at any study interval.

7.5. Protein

Protein intake increased in the LCD (98.8, 111.5, 109.4, 110.2 & 110gms/day baseline, 6weeks, 3, 6 & 12 months respectively) and LGL (96.6, 107.9, 106.6, 98.1, & 102.2gms/day) groups (Table 44). These rises were expected and in concordance with the recommended dietary requirements (Table 18) reaching 30% for LCD and 25% for LGL ($p <0.001$ for both) of the total energy intake. Total protein levels fell in the LFD group (96.1, 74.1, 71, 82.8 & 81.8gms/day at the same study intervals. Although these reductions were up to 22% of initial protein intake, the overall proportion of calories from protein remained stable (18-20%) and above the recommended dietary target of 10-15%.

The changes in protein intake was adequate to maintain a significant difference throughout the study intervals between LCD and LFD (all $p<0.05$), LGL and LFD at 6 weeks and 3 months ($p<0.05$), but no difference was noted for LCD and LGL at any stage.

7.6. Carbohydrate

The average carbohydrate intake at the start was 225gms/day (Table 44) and approximately 40% of total daily calories. At baseline refined sugars made up approximately 40% (84.2, 95.7, 100.7gms/day for LCD, LFD & LGL respectively), of the carbohydrate load. The LCD group reduced its carbohydrate intake by up to 80% as recommended in the dietary advice and maintained the reductions for 3 months after which incremental increases were made (220.4, 40.2, 42.5, 61.7, & 114.0gms/day at baseline, 6weeks, 3, 6 & 12 months) as subjects gradually increased the carbohydrate intake. Reductions occurred in the other two arms but not to as extreme levels (12.1, 11.2, 9.5 & 17.5%, LFD; 27.9, 29.4, 27.9 & 17%; LGL) at 6weeks, 3, 6, & 12 months respectively) (Figure 24).

Carbohydrates were to make up 50-60% of the total daily energy intake for the LFD group and 40% for the LGL group. Both groups remained below these targets. The LFD group achieved 46.5, 47.4, 46.9 & 43.3% at 6 weeks, 3, 6 & 12 months. The LGL group initially reduced intake to 33.6% at 6 weeks but values then rose to 35.7, 36.8 & 37.5%.

Although the total amount of refined sugar intake fell in all three groups (84.2, 19.5, 23.1, 29.7 & 50.8gms/day for LCD; 95.7,81.3, 80.4, 84.3 & 66.9gms/day for LFD; 100.7, 74.7, 71.4, 80.8, & 87.1gms/day for LGL at baseline, 6 weeks, 3, 6 & 12 months, the proportion of refined sugar making up the total carbohydrate intake rose in both the LCD (38.8, 55.8, 57.9, 53.6 & 51.9%) and LGL (42.3, 49.4, 45, 49.3& 48.2%) arms, but remained static in the LFD (41.6, 41.5, 42.6 39.6 & 39.7) group. This is in direct contrast to the recommendations of both the LCD and LGL diets which encourage the use of complex carbohydrates with low glycaemic loads. Changes in refined sugar intake were significant between the LCD and LFD group at 6 weeks, 3 & 6 months ($p<0.01$), the LCD and LGL group at all study intervals ($p<0.01$) but not between LGL and LFD at any study interval.

Figure 24: Changes in total calories and calories from each source by percentage across the three groups

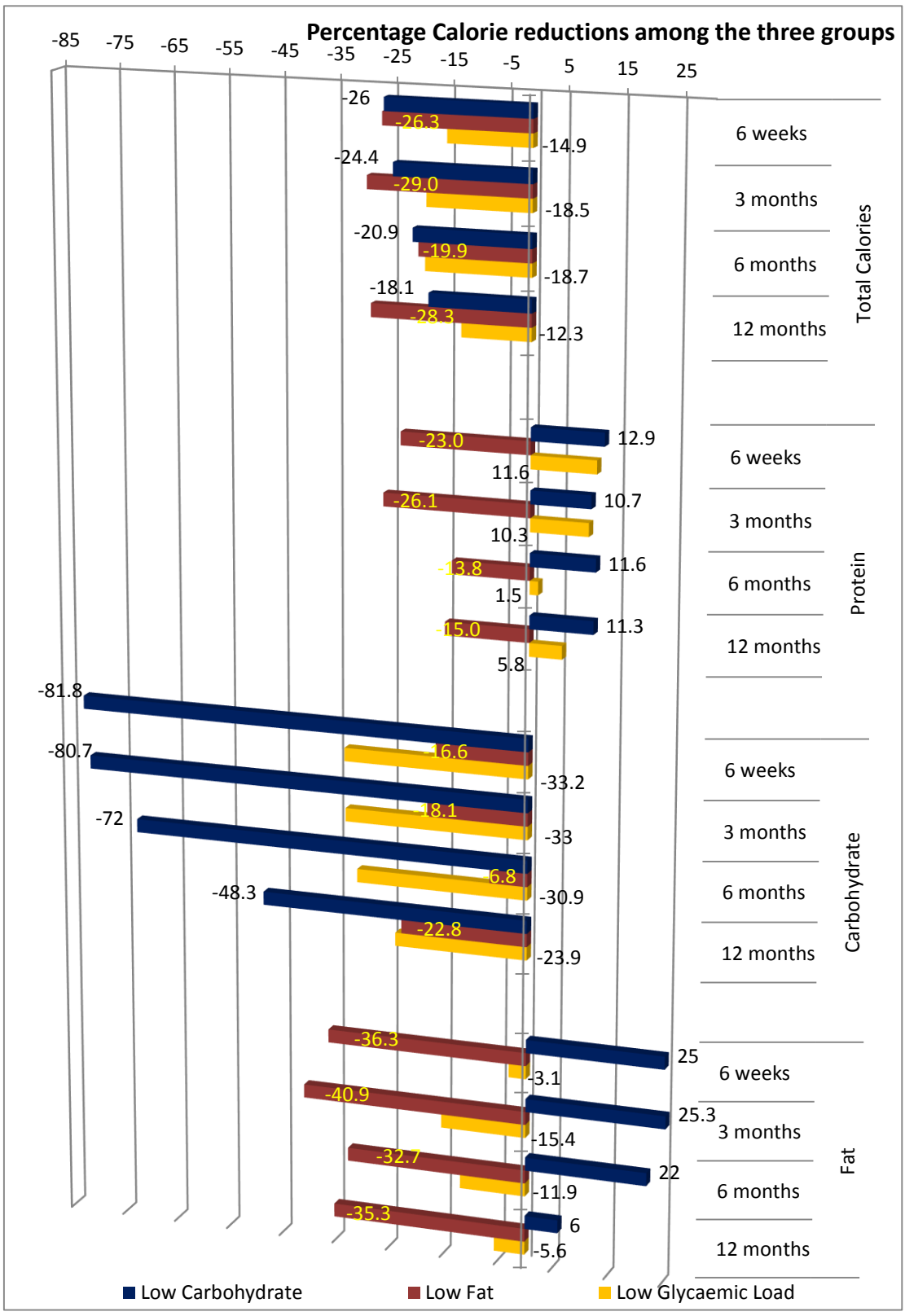


Table 43: Variations in caloric intake among the three diets

	Low Carbohydrate		Low Fat		Low Glycaemic Load	
	Daily Average	Percentage of energy	Daily Average	Percentage of energy	Daily Average	Percentage of energy
Total Average Caloric Intake						
Baseline	1998.5± 670.5		2079.8± 641.5		2007.5± 393.1	
6 weeks	1479.8± 435.3*		1532.9± 429.9♥		1709.2± 266.1♦	
3 months	1510.6± 346.3*		1477.0± 412.3♥		1636.5± 301.9*	
6 months	1580.6± 444.9*		1665.5± 316.7*		1632.2± 351.7*	
12 months	1635.9± 434.1		1491.9± 393.3*		1760.5± 409.7	
Protein						
Baseline	395.1± 116.0	20.0± 4.4	384.6± 122.4	18.4± 3.2	386.5± 99.2	18.8± 3.2
6 weeks	446.0± 166.1♥	30.1± 7.2	296.2± 111.4	18.6± 3.5	431.3± 82.1♥	24.9± 4.2
3 months	437.5± 132.7♥	28.8± 6.3	284.1± 87.8	18.8± 3.1	426.2± 115.2♥	25.4± 4.9
6 months	440.8± 139.5♥	27.9± 6.1	331.4± 81.3	19.6± 4.2	392.2± 91.0*	23.9± 4.7
12 months	439.8± 111.9♥	27.5± 7.1	327.0± 80.2	21.8± 3.9*	408.8± 95.5♥	22.8± 3.3
Carbohydrate						
Baseline	826.5± 364.5	40.8± 12.0	855.5± 288.2	40.8± 7.6	888.0± 212.9	43.5± 6.9
6 weeks	150.8± 114.2♥	10.2± 7.3	713.8± 191.1♦	46.5± 9.9	593.2± 175.2*	33.6± 8.7
3 months	159.5± 125.2♥	10.5± 7.6	700.3± 161.0♦	47.4± 9.4	595.2± 138.8*	35.7± 6.5
6 months	231.3± 161.0♥	14.1± 9.2	797.0± 166.4♦	46.9± 6.5	613.4± 146.9♥	36.8± 4.6
12 months	427.5± 271.4♥	24.0± 12.5	660.1± 233.5	43.3± 9.8	675.6± 184.3♦	37.5± 6.8
Fat						
Baseline	688.6± 308.2	33.1± 9.2	680.6± 207.5	32.4± 5.6	672.8± 176.3♦	32.7± 4.5
6 weeks	860.5± 306.9♥	56.7± 6.8	433.3± 139.5	27.6± 5.9*	651.9± 147.8	37.6± 8.1
3 months	863.0± 260.8♥	55.6± 9.2	402.4± 156.0	26.3± 6.5*	569.3± 129.5	34.0± 5.1
6 months	839.8± 304.6♥	51.7± 8.6	458.0± 123.3	27.0± 6.2*	592.8± 173.3	35.3± 4.6
12 months	730.0± 227.9♥	43.7± 7.3	440.7± 169.9	28.9± 8.9	635.1± 204.6	35.2± 7.7

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values are for within group comparisons back to baseline)

Table 44: Changes in the major nutritional components throughout the study phase

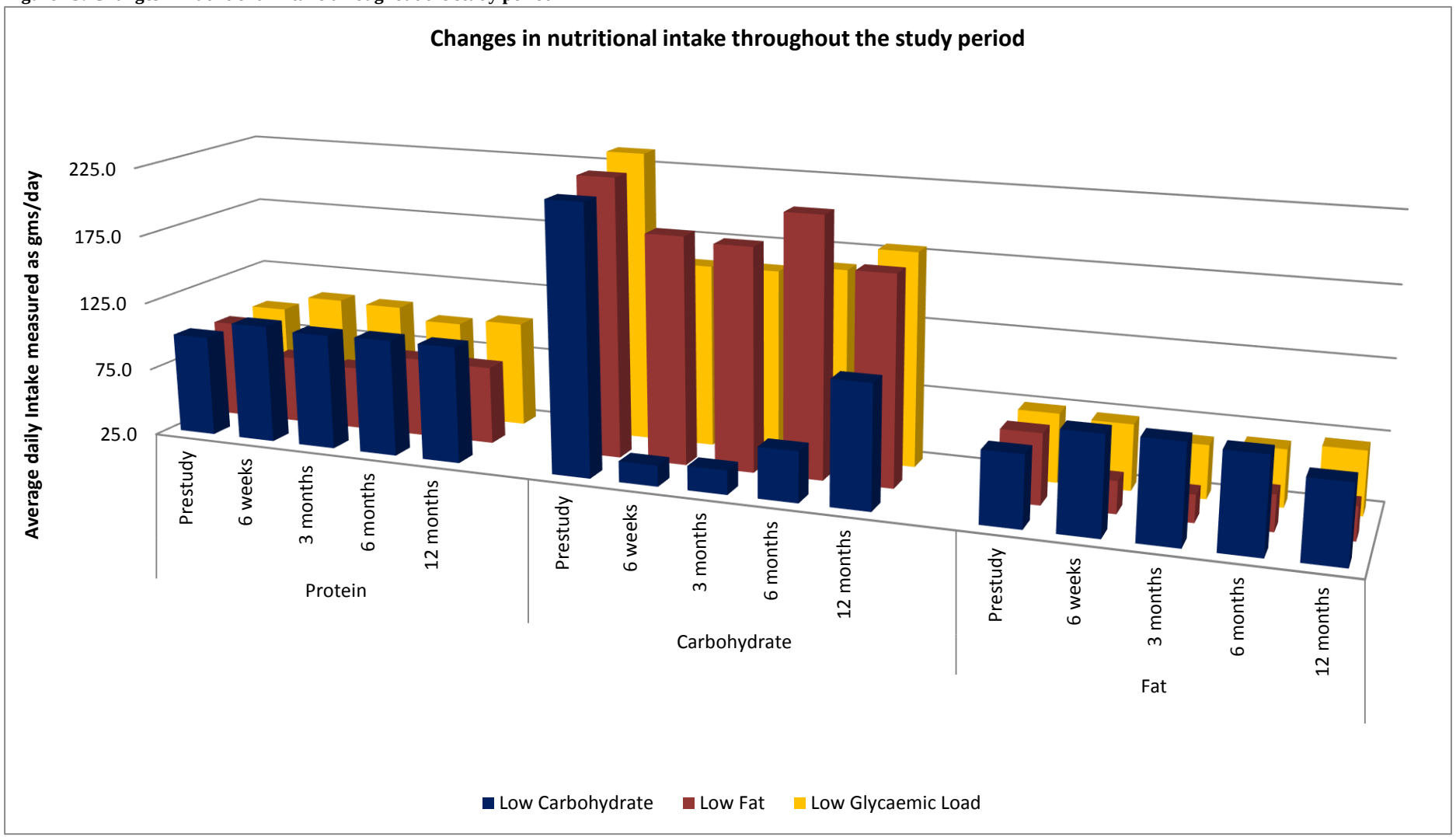
	Low Carbohydrate	Low fat	Low Glycaemic Load
Protein (gms)			
Baseline	99 ± 29	96 ± 31	97 ± 25
6 weeks	112 ± 42♦	74 ± 28♥	108 ± 21
3 months	109 ± 33	71 ± 22♥	107 ± 29
6 months	110 ± 35	83 ± 20♦	98 ± 23
12 months	110± 28.0	81± 20.1	102± 23.9
Carbohydrate (gms)			
Baseline	220 ± 97	228 ± 77	237 ± 57
6 weeks	40 ± 31♥	190 ± 51♦	158 ± 47♥
3 months	42 ± 33♥	187 ± 43♦	159 ± 37♥
6 months	62 ± 43♥	213 ± 44	164 ± 39♥
12 months	114 ± 72♥	176 ± 62♦	180 ± 49♦
Fat (gms)			
Baseline	77 ± 34	76 ± 23	75 ± 20
6 weeks	96 ± 34	48 ± 16♥	72 ± 16
3 months	96 ± 29♦	45 ± 17♥	63 ± 14♦
6 months	93 ± 34	51 ± 14*	66 ± 19
12 months	81 ± 25	49 ± 19*	71 ± 23
Refined Sugars (gms)			
Baseline	84 ± 42	96 ± 43	101 ±32
6 weeks	20 ± 16♥	81 ± 37	75 ±22*
3 months	23 ± 17♥	80 ± 30	71 ±26*
6 months	29.7± 21.9♥	84.3± 25.1	80.8±25.7♦
12 months	50.8± 32.7*	66.9± 24.4♦	87.1±27.7
Fibre (gms)			
Baseline	20 ± 8	21 ± 8	22 ± 7
6 weeks	9 ± 3♥	21 ± 6	19 ± 8
3 months	11 ± 4♥	20 ± 7	20 ± 6
6 months	12 ± 10*	22 ± 5	18 ± 6♦
12 months	17.4± 9.2♦	18.2± 7.8	21.0± 8.5
Saturated Fat (gms)			
Baseline	29 ± 15	27 ± 10	27 ± 7
6 weeks	37 ± 14♦	15 ± 6♥	24 ± 7
3 months	38 ± 13	15 ± 6♥	21 ± 6*
6 months	37 ± 17	17 ± 7*	21 ± 6♦
12 months	29.2± 9.5	16.0± 6.2*	22.6± 8.3
Monounsaturated Fats (gms)			
Baseline	27 ± 12	26 ± 8	26 ± 8
6 weeks	35 ± 13♦	18 ± 8♥	27± 6
3 months	36 ± 12*	15 ± 9♥	24 ± 7
6 months	33 ± 13	18 ± 5*	24 ± 8
12 months	29 ± 10	17 ± 7*	26 ± 10

Table 45: Changes in the major nutritional components throughout the study phase cont…

	Low Carbohydrate	Low fat	Low Glycaemic Load
Polyunsaturated Fats (gms)			
Baseline	13 ± 6	12 ± 5	13 ± 5
6 weeks	14 ± 7	10 ± 5♦	14 ± 6
3 months	13 ± 6	9 ± 4♦	11 ± 4
6 months	13 ± 4	9 ± 3♦	12 ± 6
12 months	14 ± 8	10 ± 6	13 ± 4♦
Cholesterol (mgs)			
Baseline	348 ± 149	392 ± 195	312 ± 134
6 weeks	670 ± 247♥	185 ± 111*	369 ± 164
3 months	655 ± 289♥	197 ± 124*	347 ± 228
6 months	611 ± 233*	231 ± 178♦	293 ± 92♦
12 months	493 ± 198. *	228 ± 95*	359 ± 138
Sodium (mgs)			
Baseline	3115 ± 1329	3070 ± 1014	3157 ± 917
6 weeks	2822 ± 1209	2387 ± 1124*	2801 ± 1060
3 months	2840 ± 1433	2554 ± 1057♦	3182 ± 1529
6 months	3004 ± 1435	2652 ± 933♦	2867 ± 943
12 months	3488 ± 1285	2495 ± 1104♦	3022 ± 1073
Calcium (mgs)			
Baseline	889 ± 436	881± 217	944 ± 286
6 weeks	623 ± 271*	726 ± 244*	1005 ± 272
3 months	522 ± 214♥	701 ± 120*	941 ± 303
6 months	642 ± 367♦	801± 185	878 ± 247
12 months	700 ± 338	773 ± 294	928 ± 253

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to within group changes from baseline)

Figure 25: Changes in nutritional intake throughout the study period



7.7. Fat

A significant reduction in fat intake was noted in the LFD group (75.6, 48.2, 44.7, 50.9 & 49gms/day) $p<0.01$) across the 5 study intervals (baseline, 6weeks, 3, 6 & 12 months) (Table 45, Figure 25). Total fat intake did not change significantly in the LGL group (74.8, 72.4, 63.3, 65.9, & 70.6gms/day). Although levels rose among the LCD group in concordance with the diet requirements (76.5, 95.6, 95.9, 93.3 & 81.1gms/day), the changes were only significant at 3 months ($p<0.05$).

When looking at targets, all three groups achieved the recommended percentage of energy intake as fat, (LCD 56.7%, LFD 27.6%, and LGL just above the required 35% with 37.6%) at 6 weeks. They maintained these proportions with the exception of LCD which dropped to 43.7% at 12 months.

Fat intake was also analysed depending on fat sub-fractions as these were expected to be somewhat different among the three study arms. The LCD group who had the highest fat intake were expected to have equal intake from saturated, monounsaturated and polyunsaturated fats. For the LFD group saturated fat intake was restricted to less that 30% of total fat intake (<10% total calories) with the remainder of the fat intake divided between monounsaturated and polyunsaturated fats with no allocated ratios. The LGL group had a higher total fat intake allowance than LFD but similar restrictions on saturated fat. Monounsaturated fats were prescribed to be the main source of fats with polyunsaturated fats, particularly omega 3 forming the rest.

In keeping with the changes seen in overall fat intake, saturated, monounsaturated and polyunsaturated fat intake rose in the LCD group and fell in the LFD group across the board Figure 27 & Table 46). Saturated fat levels fell in the LGL group but monounsaturated and polyunsaturated levels remained stable. The changes in saturated, monounsaturated and polyunsaturated fat were significant for all stages in the LFD group ($p<0.01$), reached significance in the LCD group for monounsaturated fats at 6 weeks and 3 months ($p<0.05$), and the LGL group for saturated fats at 3 & 6 months ($p<0.05$). Comparing the groups the differences were significant between LCD & LFD ($p<0.01$) at 6weeks, 3 and 6 months, and LCD & LGL ($P<0.05$) at 6weeks, 3 and 6 months.

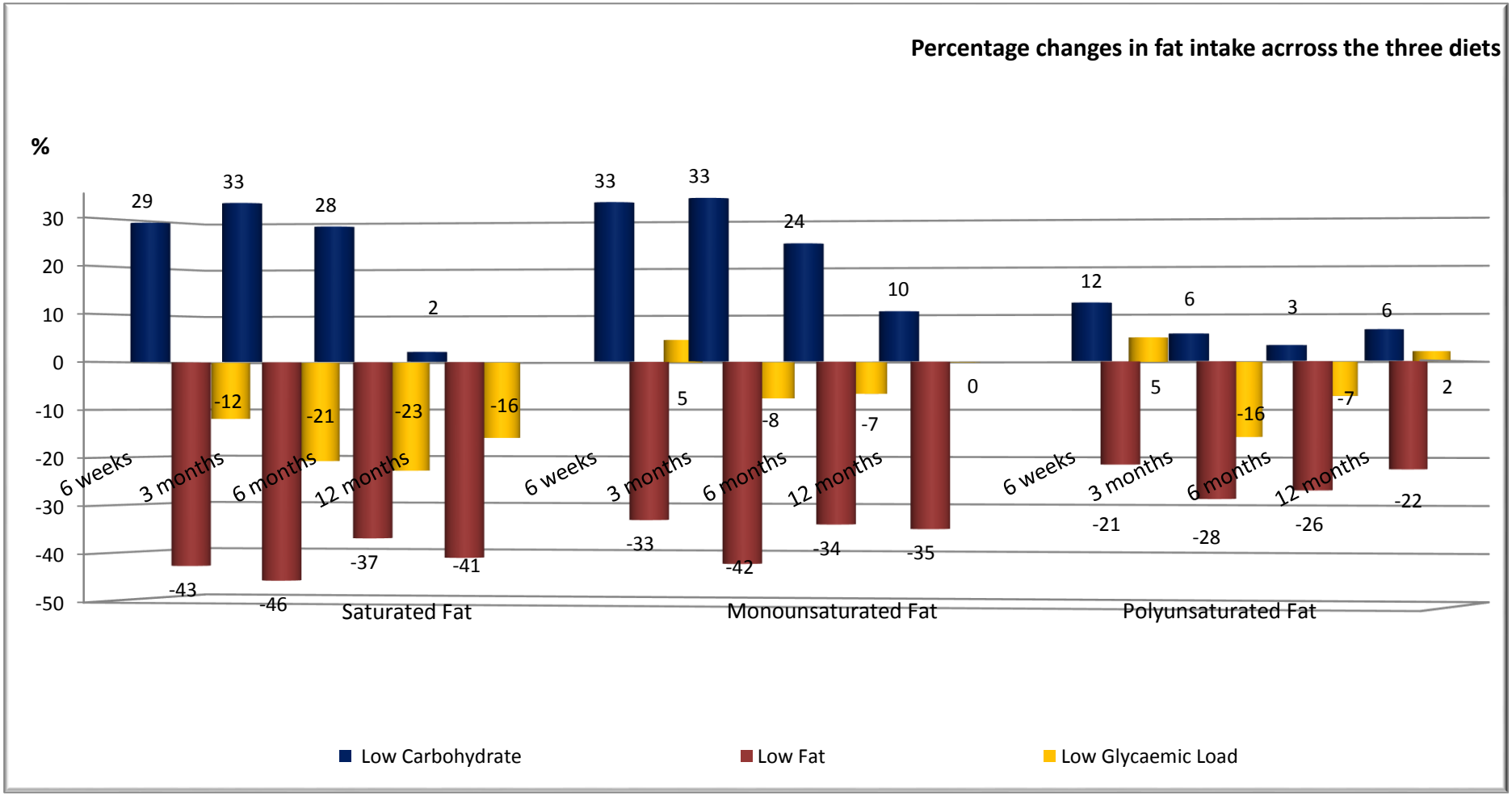
Table 46: Fat sub-fractions as a percentage of total fat daily intake among the three groups

	Low Carbohydrate	Low Fat	Low Glycaemic Load
Saturated Fat (%)			
Baseline	36.4±6.2	35.5±5.2	36.2±5.9
6 weeks	38.8±5.2	32.0±6.3♦	32.9±6.0
3 months	39.4±5.2	33.0±5.3♦	33.5±5.9
6 months	38.4±6.7	33.1±5.0♦	31.8±5.1♦
12 months	36.5±5.3	33.2±6.2	32.2±5.4♦
Monounsaturated Fat (%y)			
Baseline	34.5±3.3	35.1±3.2	34.1±3.1
6 weeks	36.9±3.0	36.2±6.3	37.4±3.4♦
3 months	36.9±5.0	33.3±6.7	37.4±5.2
6 months	35.5±4.9	34.6±5.3	36.7±5.3
12 months	36.2±4.0	34.9±3.8	36.1±4.3
Polyunsaturated Fat (%)			
Baseline	16.9±3.9	16.5±4.8	17.2±3.7
6 weeks	14.5±3.8	20.3±6.9	18.5±4.9
3 months	13.9±4.0	20.3±5.9	17.0±3.7
6 months	14.9±5.0	18.1±3.9	18.2±5.0
12 months	16.1±6.8	18.9±5.9	19.1±4.4

♦ = $p<0.05$ - **(p-values refer to within group changes from baseline)**

Cholesterol intake was calculated from the dietary records at baseline, 6 weeks, 3, 6 and 12 months. The LCD group almost doubled cholesterol intake within the first six months (348, 670, 655, 611 & 493mgs/day; $p<0.001$ at 6, 12, & 24weeks) before moving back towards baseline. Levels in the LFD group fell reaching the lowest at six weeks before gradually rising (393, 185, 197, 231 & 228mg/day, all $p<0.01$). Cholesterol levels in the LGL group remained stable (312, 369, 347, 293, and 359mgs/day; all p=NS). Changes in cholesterol intake were adequate to maintain a difference between LCD and LFD (all $p<0.01$) as well as LCD and LGL (all $P<0.05$) at all study intervals, and LFD and LGL at 6weeks, and 12 months ($p<0.05$).

Figure 26: Percentage change in fat sub-group intake across the three interventions



7.8. Salt

The average salt intake among the three groups was 3100mg/day. In the LFD group sodium levels fell by 22.2% at 6 weeks ($p<0.01$) and remained low to the end of the study period (9.7, 17.5 & 11.1% at 3, 6 & 12 months; all $p<0.05$). Salt intake remained level in the LGL group. Salt intake within the LCD group was stable for the first six months (0.3, 3.9 & 1.2% at 6 weeks, 3 & 6 months; p=NS) until the end when salt intake appeared to have increased by 24.3%. This change was not statistically significant. At no stage were there any significant changes between the groups.

7.9. Calcium

Changes in calcium was most prominent in the LCD group where levels fell by 25, 32, 24 & 21% at 6 weeks, 3, 6 & 12 months ($p<0.01$ at 6 & 12 weeks, and <0.05 at 6 months). A fall in calcium intake was also observed in the LFD group with levels dropping by 17, 20, 9 & 11% ($p<0.01$ at 6 & 12 weeks) for the study intervals. Within the LGL group calcium levels initially rose by 7% (not significant) at 6 weeks then returned to baseline. Changes between groups were significant for LCD vs. LGL at 6 weeks ($p<0.05$) and 6 months ($p<0.01$).

7.10. Potential Inaccuracies within the Food Diaries

Although food diaries can be useful to help provide a log of dietary intake which could be fed into a computer programme to generate an objective analysis of caloric and nutritional intake, there are several disadvantages which are seen in most studies using food diaries or recollection for data collection. The first of these being a misrepresentation of information supplied within the food diaries. Food diaries can help collate a picture of food intake over a number of days, but as the subjects

collating the information are conscious of the exercise there tends to be a biased record of intake as individuals consciously choose healthier options. An example is the alcohol intake which was poorly documented within the majority of diaries. It is uncertain whether this is due to lack of regular intake, a conscientious lack of intake during the monitoring period, or a lack of appreciation that alcohol may have caloric or nutritional significance. Another example is the salt intake. Current recommendations are up to 6g of salt per day which is the equivalent of a full teaspoon. The average intake at the start of the study was 3g per day, half the recommended levels.

The second issue is the accuracy of the data supplied, as this is subject to individual's ability to correctly weigh and estimate the various food items. Some have argued that absolute figures are not necessary as long as the data collated represented equal proportions of the entire food spectrum. This argument was for epidemiological studies where data is usually collated through 24 hour recall or non-weighed food diaries.

In the majority of the diaries the daily average caloric intake appeared to be relatively lower than what was prescribed for those subjects allocated to energy restricted diets. Weight loss in a number of these individuals was certainly not at the rate that would be expected for the degree of calorie restriction raising the question of under-estimating intake or deliberate caloric reductions during the weighed food diary exercise. Directly challenging the subjects is unlikely to provide any improvements in reducing these biases. Only methods to improve this would be through running dietary trial studies in areas where the individuals were institutionalized and supplied with pre-prepared meals whose nutritional and caloric intake were calculated.

It is possible to estimate the expected weight loss for most individuals and therefore roughly assess the degree of under-reporting within the food diaries. Estimation of daily caloric requirements can be made through the calculation of the basal metabolic rate. This is a measurement of the daily energy expenditure of a human when at rest. Measurements can be made through a formula which was originally created in 1919 and named the "Harris-Benedict equation". This formula was

For men,

$$P = \left(\frac{13.7516m}{1\ \text{kg}} + \frac{5.0033h}{1\ \text{cm}} - \frac{6.7550a}{1\ \text{year}} + 66.4730\right)\frac{\text{kcal}}{\text{day}}$$

For women,

$$P = \left(\frac{9.5634m}{1\ \text{kg}} + \frac{1.8496h}{1\ \text{cm}} - \frac{4.6756a}{1\ \text{year}} + 655.0955\right)\frac{\text{kcal}}{\text{day}}$$

***P* = total heat production at rest, *m* = weight, *h* = height, *a* =age[223].**

Over time the equations were modified to account for lean body mass, lifestyle changes, and levels of activity. Schofield's equations were introduced in 1980s and adopted with some modifications by the Joint Food and Agricultural Organization/World Health Organization/United Nations University (FAO/WHO/UNU) to be used [475]. These were based on a meta- analysis of over 100 European and North American studies. Individuals' daily nutritional requirements were calculated based on a series of factors including gender, weight, height, occupation, and level of activity. They have generally been adopted for use in numerous dietary studies although they have been criticised for overestimating the basal metabolic rate among Eastern individuals (Indian and East Asian). Consequently some modifications were made with the production of the Oxford Brookes Basal Metabolic Rate database[233].

Calculating a subject's basal metabolic rate provides a figure against which an estimate of how much caloric intake will affect weight. Those with grossly underestimated caloric intake, but no compatible weight loss are thus likely to be subjects who were selective with their dietary intake on the food diary data collection days, or were intentionally or unintentionally missing recording items or the portion sizes. To note these equations are predictive values and not an accurate measure of the subject assessed.

8. CHAPTER 8: The Effect of the Three Dietary Regimens on Insulin Resistance

8.1. Changes in Glucose

Fasting glucose was measured at the four study intervals, baseline, 3, 6 and 12 months, but a glucose tolerance test was only performed at baseline, 6 and 12 months. Fasting and 2 hour glucose levels fell among all three dietary arms with changes sustained till the end of the 12 month study period. Fasting glucose levels fell by 5, 7 & 6% (all p=NS) for LCD, by 7, 7& 4% ($p<0.01$ at 3 & 6months) for LFD, and by 6, 6 & 8% ($p<0.05$ at 3 & 12 months) for LGL. Post-prandial (2 hour post OGTT) reductions at 6 & 12 months were 15 & 13% (all $p<0.05$) for LCD, 18 & 6% ($p<0.01$ at 6months) LFD, and 6 & 19% (all $p<0.01$) for LGL.

The number of individuals with diabetes was small and glycosylated haemoglobin was only measured in those with diabetes. There was a trend for HbA1c levels to fall among those with diabetes in all three groups, but reductions were only statistical significant at three months in the LGL group ($p<0.05$) (Table 47).

8.2. Changes in Insulin levels

In keeping with reductions in glucose levels, there was an observed fall in insulin levels among the three study groups at all study intervals with reductions of 23, 20 & 29% (all $p<0.01$) for LCD, 14, 22, & 15% (all $p<0.01$) for LFD, and 19, 18 & 30% (all $p<0.01$) for LGL at 3, 6 and 12 months. At no point in the study were there any differences between the groups for glucose, HbA1c, insulin levels or insulin resistance (Table 47).

Table 47: Changes in Glucose and Insulin levels

	Low Carbohydrate	Low fat	Low Glycaemic Load
Fasting glucose mmol/L			
Baseline	5.8± 1.9	5.9±1.5	5.99±1.8
3 months	5.3±0.8	5.4±1.0♥	5.57±1.2♦
6 months	5.1±0.73	5.3±0.8*	5.8±2.04
12 months	5.1±0.8	5.6±1.2	5.2±2.2*
2 hr Glucose (GTT) mmol/L			
Baseline	7.6±4.1	8.1±4.2	8.0±4.0
6 months	5.3±1.5♦	6.2±2.5♥	6.7±3.3*
12 months	5.4±1.7♦	6.9±3.1	6.0±4.2♥
HbA1c %			
Baseline	6.7±1.4	7.0±0.9	6.6±0.8
3 months	5.9±0.8	6.2±0.4	6.2±0.7♦
6 months	5.6±0.4	6.1±0.3	6.3±0.8
12 months	5.6±0.4	6.3±0.6	6.6±1.1
Insulin levels mIU/ml			
Baseline	22.8±14.9	18.6±9.9	19.6±11.1
3 months	16.3±8.3♥	14.8±8.5♥	15.0±9.3*
6 months	16.0±10.6♦	13.3±6.2♥	13.8±7.0*
12 months	13.8±6.8♥	14.4±7.0*	12.2±7.0♥
HOMA IR			
Baseline	2.9±1.7	2.4±1.3	2.5±1.4
3 months	2.1±0.1♥	1.9±1.1♥	1.9±1.2*
6 months	2.0±1.3♦	1.7±0.8♥	1.8±0.9*
12 months	1.7±0.9*	1.8±0.9*	1.7±0.9♥
HOMA %B			
Baseline	154.5±79.1	133.4±54.0	134.21±65.01
3 months	140.9±51.4♦	129.5±51.9	123.06±56.48
6 months	145.4±52.1	118.6±31.7♦	123.3±55.1
12 months	133.3±51.4♦	118.9±46.0	112.8±46.3*

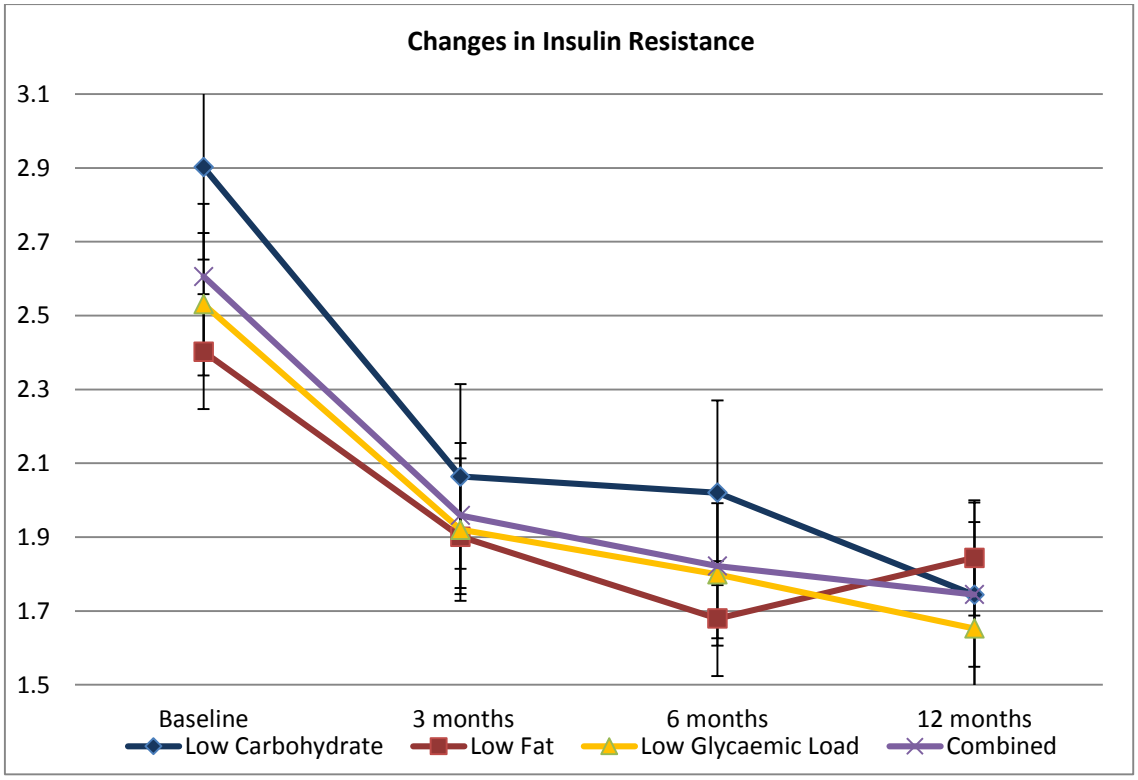
♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to within group changes from baseline)

8.3. Changes in Insulin Resistance

Improvements in insulin resistance were noted in all three dietary interventions (Table 47) with insulin levels falling with improvements in insulin resistance. All three diets achieved significant reductions in insulin resistance with levels falling by 23, 24 & 28% (all $p<0.01$) for LCD, 19, 23 & 16% (all $p<0.01$) for LFD, and 20, 18 & 28% (all $p<0.01$) for LGL. Reductions were maintained in both LGL and LCD groups (Figure 27). No significant changes were noted between the groups at any point.

Improvements in insulin sensitivity were present throughout the study period and significant for each group apart from LFD at 3 months where the insulin sensitivity figures appear to be disproportionately elevated. Improvements in calculated ß-cell function similarly reflected the changes seen in insulin resistance and sensitivity.

Figure 27: Reduction in Insulin Resistance (HOMA-IR)



Graph demonstrates the changes in insulin resistance at each **study interval with standard error**

8.4. Correlations of Weight to Insulin and Insulin Resistance

When analysed as a cohort the reduction in weight positively correlated to fasting glucose levels (r= 0.37, 0.23, 0.24; $p<0.05$) at start, 6 & 12 months, and insulin levels (r= 0.44, 0.40, 0.50, & 0.37; all $p<0.001$), insulin resistance (r= 0.45, 0.37, 0.50 & 0.39; all $p<0.001$), ß-cell function (r= 0.12, 0.22, 0.22, 0.15; $p<0.05$ at 3 & 6 months), and negatively correlated to insulin sensitivity (r= -0.32, -0.10, -0.40, & -0.28; $p<0.05$ at 0, 6 & 12 months) at baseline, 3, 6 & 12 months.

Waist measurements also demonstrated similar positive correlations to fasting glucose (r= 0.40, 0.15, 0.26, & 0.27; $p<0.05$ at 0, 6 & 12 months), insulin (r= 0.44, 0.46, 0.53 & 0.54; all $p<0.001$), insulin resistance (r= 0.45, 0.44, 0.53, & 0.55; all $p<0.001$), ß-cell function (r= 0.13, 0.30, 0.28, & 0.26; $p<0.05$ at 3, 6 & 12 months), and a negative correlation to insulin sensitivity (r= -0.41, -0.91, -0.44, & -0.41; $p<0.001$ at 0, 6 & 12 months) at baseline, 3, 6 & 12 months respectively.

Despite a significant reduction in glucose, insulin levels, improvements in insulin resistance and calculated ß-cell function, these effects did not correlate with weight loss for either LGL or LFD. No correlation for weight loss and fasting glucose was observed for the LCD group but a strong positive correlation was noted between weight and insulin levels (r =0.68, 0.60, 0.60 & 0.62, all $p<0.001$) insulin resistance (r = 0.67, 0.53, 0.58 & 0.61, all $p<0.01$) and ß-cell function (r =0.36, 0.52, 0.41 & 0.48 all $p<0.05$), and a negative correlation to insulin sensitivity (r= -0.60, -0.53, -0.59 & -0.59, all $p<0.01$) at baseline, 3, 6 & 12 months respectively.

Similarly strong correlations were noted for the LCD group for waist circumference and insulin levels (r= 0.72, 0.6, 0.64 & 0.71, all $p<0.001$), insulin resistance (r= 0.72, 0.61, 0.63 & 0.69, all $p<0.001$) and ß-cell function (r= 0.35, 0.50, 0.49 & 0.56, $p<0.05$), and a negative correlation to insulin sensitivity (r=-0.64, -0.56, -0.57 & -0.59, $p<0.01$) at baseline, 3, 6 & 12 months respectively.

In the LGL group, waist measurements also correlated to insulin (r=0.28, 0.45, 0.53 & 0.56; $p<0.01$ at 3, 6 & 12 months), insulin resistance (r= 0.32, 0.44, 0.55

& 0.58; all p<0.01), and negatively to insulin sensitivity (*r*= -0.47, -0.35, -0.55 & -0.53; all p<0.01) at baseline, 3, 6 & 12 months respectively. No correlation was demonstrated for ß-cell function.

No correlations to waist measurements were demonstrated in the LFD group.

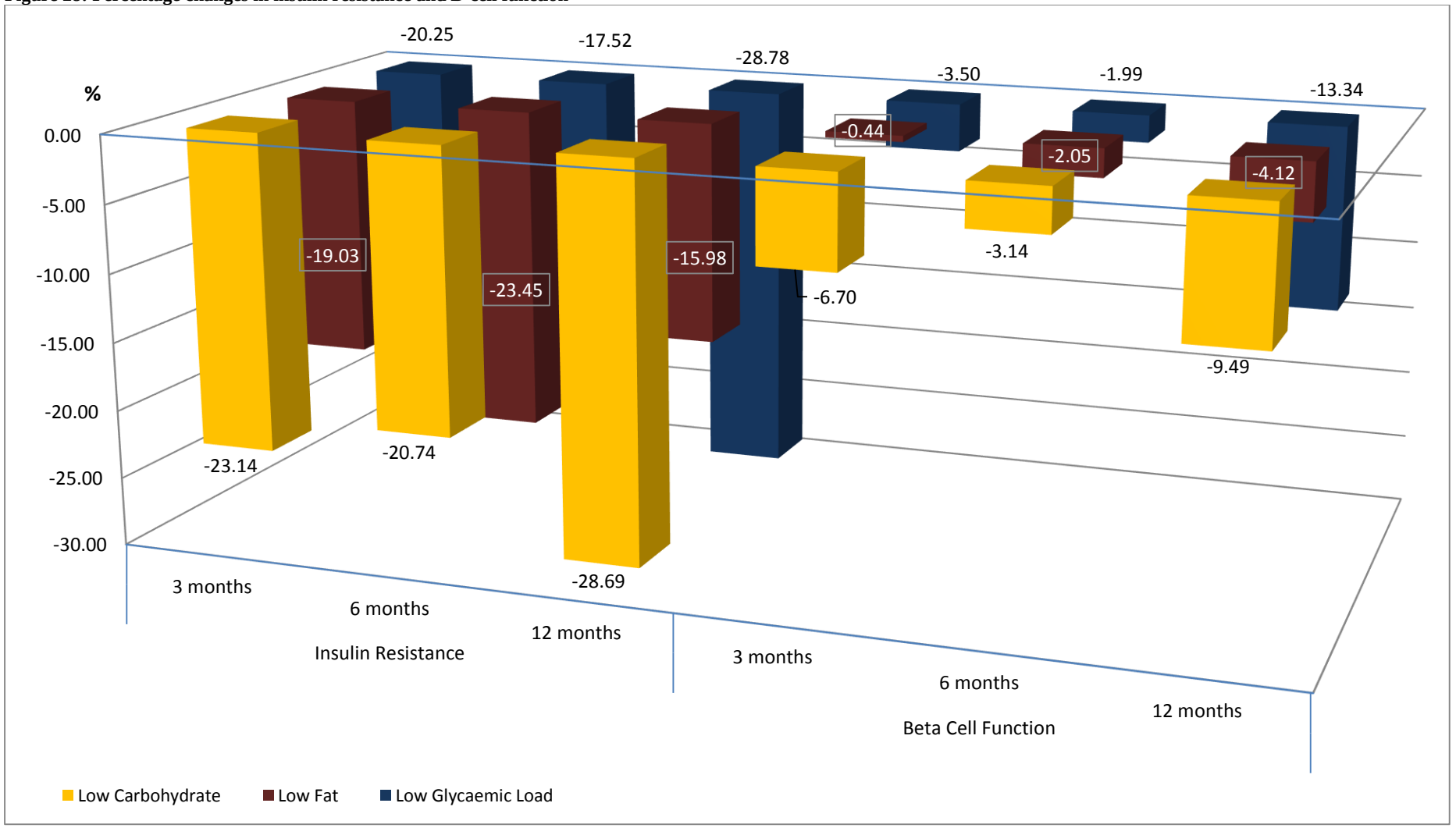
When looking at correlations of fasting glucose to insulin and insulin resistance in the study cohort, these positively correlated to insulin (*r*= 0.31, 0.29, 0.32 & 0.25; all p<0.05) and insulin resistance (*r*= 0.45, 0.37, 0.41, & 0.36; all p<0.01), and negatively correlated to ß-cell function (*r*= -0.55, -0.44, -0.44, & -0.49; all p<0.001), and insulin sensitivity (*r*= -0.37, -0.22, -0.37, & -0.29; all p<0.05) at baseline, 3, 6 & 12 months respectively .

Unlike the correlations demonstrated for weight and waist measurement, the strongest correlations for fasting glucose was noted among the LFD group. Fasting glucose positively correlated to insulin (*r*= 0.42, 0.38, 0.67, 0.39; all p<0.05), insulin resistance (*r*= 0.52, 0.46, 0.72, & 0.50; all p<0.01) and negatively correlated to ß-cell function (*r*=-0.57, -0.41, -0.28, &-0.42; p<0.05 at 0, 3, & 12 months) and insulin sensitivity (*r*= -0.36, -0.28, -0.55, & --0.40; p<0.05 at 0, 6, & 12 months) at baseline, 3, 6 & 12 months respectively.

In the LCD group reductions in glucose positively correlated with insulin resistance (*r*= 0.50, 0.40, 0.53, & 0.38; p<0.05 at 0, 3 & 6 months), and negatively with ß-cell function (*r*= -0.52, -.047, -0.25, & -0.45; p<0.05 at 0, 3, & 12 months) at baseline, 3, 6 & 12 months respectively. No correlations were demonstrated for fasting glucose and insulin levels and insulin sensitivity.

Fasting glucose in the LGL group was negatively correlated to ß-cell function (*r* = -0.61, -0.45, -0.56 -0.57; all p<0.01) at baseline, 3, 6 & 12 months. No correlations were seen for insulin levels, insulin resistance nor insulin sensitivity.

Figure 28: Percentage changes in insulin resistance and B-cell function



9. CHAPTER 9: The Effect of Three Dietary Interventions on Cytokine levels

9.1. Changes in Leptin

Leptin levels fell in all three groups (Table 48). Reductions were 35, 39 & 24% (all $p<0.01$) for LCD, at 15, 17 & 9% (all $p<0.05$) for LFD, and 33, 32 & 29% (all $p<0.001$) for the LGL group at 3, 6 & 12 months respectively. The lowest levels were achieved at 6 months. Differences between the groups were only present when measuring the degree of leptin reduction from baseline. These differences were between LCD and LGL ($p<0.05$) at 3 months and LCD and LFD at 3 and 6 ($p<0.01$) months.

The reduction in leptin correlated to weight in the study cohort ($r= 0.24, 0.27, 0.26$ & 0.23; all $p<0.05$) at baseline, 3, 6 and 12 months respectively and waist measurements ($r= 0.19, 0.23, 0.26,$ & 0.24; $p<0.05$ at 3, 6 & 12 months). A particularly strong correlation was seen with BMI ($r= 0.62, 0.655, 0.65$ & 0.62; all $p<0.001$)

Similar correlations were noted in the LCD group for weight ($r =0.54, 0.64, 0.54$ & 0.53; all $p<0.01$) BMI ($r = 0.76, 0.83, 0.77$ & 0.80; all $p<0.001$), and waist ($r= 0.39, 0.53, 0.45$ & 0.47; $p <0.05$ at 3, 6 & 12 months). These correlations were only identified with BMI for LFD ($r = 0.54, 0.54, 0.53$& 0.46; all $p<0.01$) and LGL ($r = 0.54, 0.62, 0.65$ &0.54; all $p<0.01$). No correlations to any of the lipid parameters were noted as a cohort or individual groups.

As leptin is associated with insulin resistance and weight loss, it was expected to see a correlation between of the markers of insulin resistance and leptin. As a study cohort leptin correlated positively with insulin levels ($r= 0.26, 0.22, 0.38,$ & 0.23; all $p<0.05$), insulin resistance ($r= 0.24, 0.21, 0.37,$ & 0.22; $p<0.05$ at baseline, 6 & 12 months), and ß-cell function ($r= 0.22, 0.33, 0.29,$ & 0.23; all $p<0.05$). Correlations to insulin sensitivity and fasting glucose were not clear.

With regards to the groups, in our study no correlation was demonstrated for fasting glucose levels, insulin resistance or ß-cell function for any of the dietary interventions with exception of the LGL group where a positive relationship was noted for insulin levels (r =0.33, 0.26, 0.69 & 0.40; $p<0.05$ at baseline, 6 & 12 months) and insulin resistance (r= 0.29, 0.27, 0.39 & 0.39; $p<0.05$ at 6 & 12 months).

Table 48: Changes in cytokine levels across the groups (leptin, adiponectin, resistin, visfatin)

	Low Carbohydrate			Low Fat			Low Glycaemic Load		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Leptin(ng/ml)									
Baseline	34.7±24.6	6.9	100.3	28.3±19.2	5.3	76.6	35.1±28.2	2.8	113.0
3 months	24.0±19.3♥	2.6	79.9	24.9±17.8*	4.1	69.6	21.3±16.1♥	2.3	80.9
6 months	22.8±19.3♥	1.8	69.5	23.1±16.9*	2.6	64.7	21.8±16.3♥	2.1	67.5
12 months	26.0±25.8*	3.6	124.1	24.9±18.7	1.0	71.3	23.8±18.4♥	1.9	81.8
Adiponectin (ng/ml)									
Baseline	7247±4242	2378	19981	10718±8647	2493	45477	6884±4438	2488	25849
3 months	8567±4313*	2874	20919	11633±10059	2832	52437	7746±5798	3063	35688
6 months	10226±518*	2254	21688	11795±10171	2428	55467	9469±8697♦	2968	50143
12 months	10192±5857♥	3582	24332	13106±10520*	3447	58074	8922±5001♥	2497	25721
Resistin (ng/ml)									
Baseline	9.0±2.0	3.2	15.5	10.1±2.6	5.7	16.7	11.6±4.9	5.4	29.6
3 months	10.0±2.9	6.8	17.5	10.2±2.5	5.9	14.6	10.8±2.9	6.8	16.0
6 months	11.0±3.2	5.7	19.3	10.7±2.8	6.2	16.3	10.7±3.1	5.1	17.6
12 months	9.9±2.2	6.4	16.0	9.7±2.4	4.7	16.5	9.6±3.2	4.3	16.6
Visfatin(ng/ml)									
Baseline	1646±2597	0	14263	1267±954	132	4325	2027±2086	109	11935
3 months	2254±2025♦	75	8525	1945±1551♦	112	5088	2366±1690	261	7218
6 months	2704±2709*	368	10038	2287±1909*	144	7975	2462±1490♦	886	8614
12 months	1685±1574♦	53	6206	1712±1349	106	5485	2203±2046	62	10471

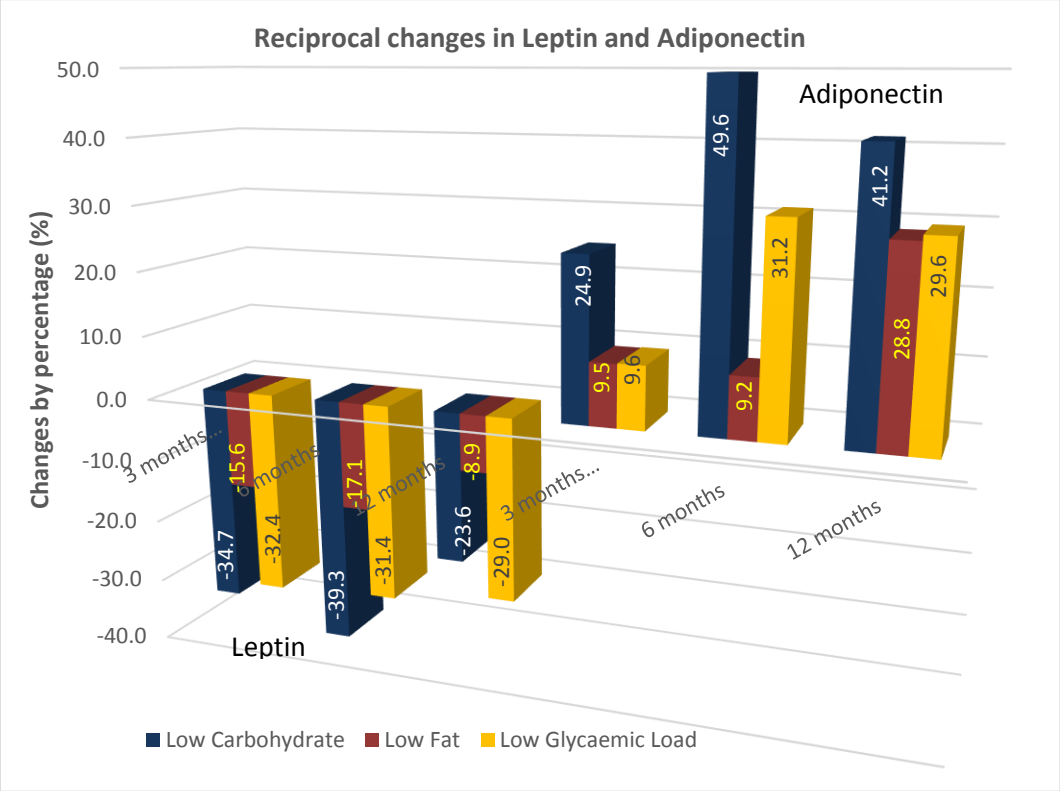
♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to within group changes from baseline)

9.2. Changes in Adiponectin

The baseline mean adiponectin level for the LFD group was higher than levels in the other two arms (Table 48). The difference was large enough to be significant between the LFD and LGL group (p=0.02) but not LFD and LCD (p=0.59) or LGL and LCD (p=1). The comparatively higher reading was maintained throughout the study although the differences between the groups was not maintained beyond the baseline.

Adiponectin levels rose within each group by 24.9, 49.6 & 41.2% (all p<0.01) for LCD, 9.5, 9.2 & 28.8% (p=NS) for LFD, and 9.5, 31.2 & 29.6% for LGL (p<0.001 at 6 & 12 month) at 3, 6 & 12 months respectively (Table 48). Maximum increases were at six months for LGL and LCD. The percentage increase in adiponectin at 6 months was significant between the LCD and LFD group (p<0.01) but otherwise no other differences were noted between the groups.

Figure 29: Reciprocal changes in Leptin & Adiponectin



Many studies investigating the effects of leptin and adiponectin have reported that an inverse relationship exists between the two [354]. The data from this study demonstrate this inverse relationship with a rise in adiponectin corresponding to the fall in leptin (Figure 29). Analysis has failed to show any form of correlation between adiponectin and leptin for the whole study cohort or any of the three dietary groups.

No correlation was present between weight, BMI or waist measurements and adiponectin for the study cohort or any of the diets.

In markers of insulin resistance, adiponectin levels did negatively correlate to insulin levels (r= -0.32, -0.32, -0.25, & -0.35; all $p<0.05$), insulin resistance (r= -0.30, --0.32, -0.24 & -0.35; all $p<0.05$) and positively to insulin sensitivity (r= 0.33, 0.13, 030 & 0.23; $p<0.05$ at baseline, 6 & 12 months) for the whole study cohort at baseline, 3 6 & 12 months respectively. No correlations for fasting glucose or ß-cell function were noted.

Within the groups adiponectin negatively correlated to insulin levels for LCD (r = -0.53, -0.49, & -0.51 at 3, 6 & 12 months; all $p<0.01$) and LFD (r= -0.43, -0.365, -0.35 & -0.44; all $p<0.05$ at 0, 3 & 12 months). A negative correlation between insulin resistance and adiponectin was noted for LCD (r = -0.54, -0.49 & -0.49; all $p<0.05$ at 3, 6 & 12 months) and LFD (r = -0.42, -0.37 & -0.43; $p<0.05$ at 0, 3 & 12 months). For ß-cell function a negative correlation with adiponectin was seen in the LCD group (r = -0.32, -0.50, -0.52 & -0.65; all $p<0.01$) for all study points. This was not noted in any of the other dietary interventions nor in the cohort analysis

Of note no correlation for adiponectin with any of the measures of insulin resistance (i.e. fasting glucose, insulin levels, insulin resistance, insulin sensitivity or ß-cell function) was evident in the LGL group.

For lipid parameters, adiponectin positively correlated to HDL-cholesterol (r= 0.49, 0.47, 0.54 & 0.45; all $p<0.001$), and negatively to triglycerides (r= -0.18, -0.34, -0.32, & -0.39; $p<0.01$ at 3, 6 & 12 months) for the whole study cohort at baseline, 3, 6 & 12 months respectively. No correlations to total or LDL-cholesterol were demonstrated.

Within the groups, as with the cohort, no correlation was established between adiponectin and total or LDL-cholesterol. Adiponectin correlated positively with HDL-cholesterol for LCD (r = 0.66, 0.65, 0.74 & 0.79; all $p < 0.001$), LFD (r = 0.51, 0.57, 0.61 & 0.60; all $p < 0.01$), and LGL (r = 0.45, 0.52, 0.54 & 0.29; $p < 0.01$ at 0, 3 & 6 months) at baseline, 3, 6 & 12 months respectively. A negative correlation was demonstrated between adiponectin and triglycerides: for LCD (r = -0.21, -0.50,-0.56 & -0.48; $p < 0.05$ at 3, 6 & 12 months), for LFD (r = -0.24, -0.47, -0.40 & -0.47; $p < 0.05$ at 3, 6 & 12 months) and for LGL (r = -0.30, -0.36, -0.28 & -0.44; $p < 0.05$ at 3 & 12 months) at the same study intervals.

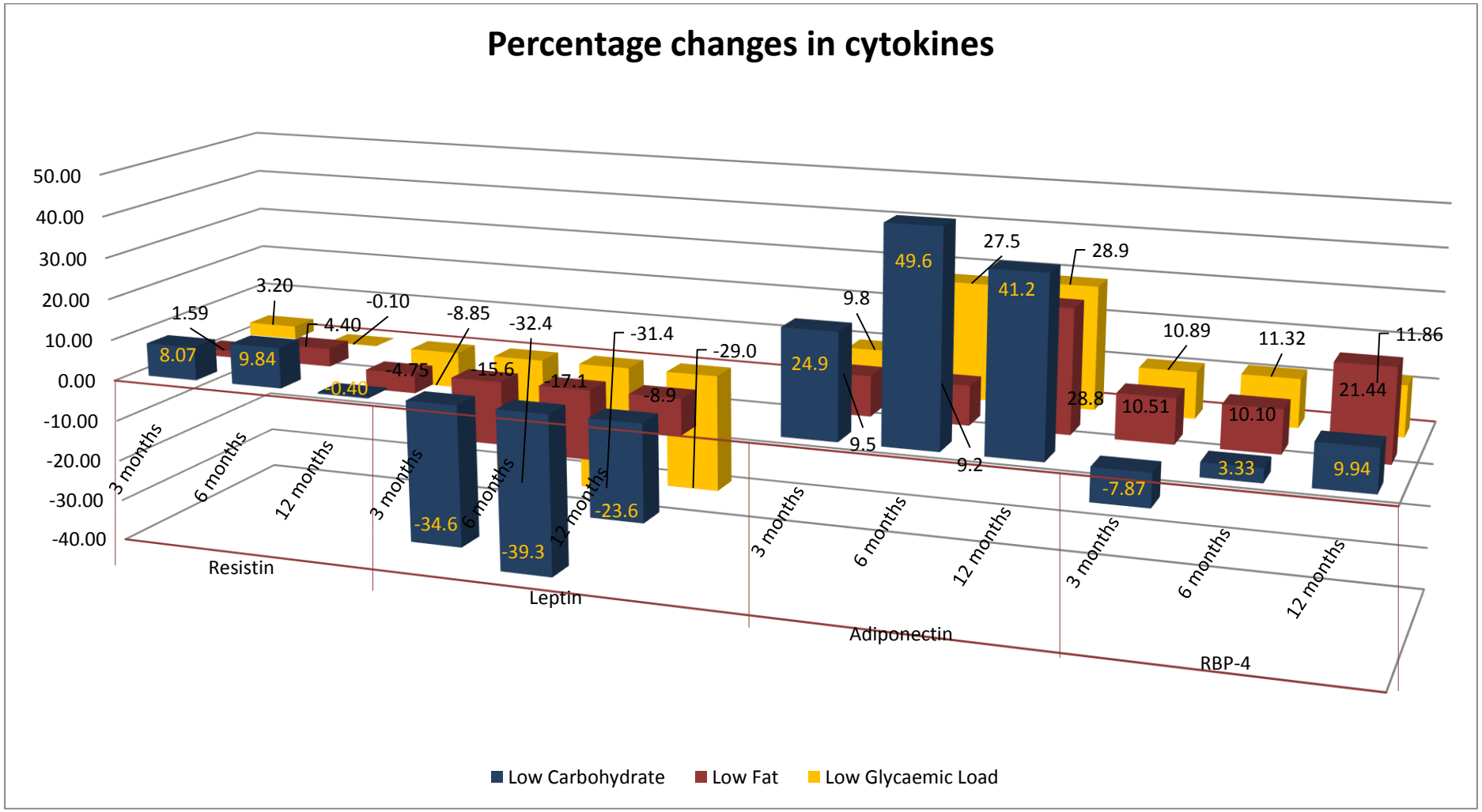
9.3. Changes in Resistin

Resistin levels varied by only small amounts in the study. Changes among LCD were -8.9, -11.5, & +0.4% (all p=NS), for LFD -1.6, -4.4, and +4.7% (all p=NS), and for LGL -0.4, +0.7 and +9.7% (all p=NS) at 3, 6 and 12 months respectively (Table 48, Figure 30). At no point in the study were there any between or within group differences.

As a study cohort no correlations were documented between resistin, anthropometric measures, lipid parameters, measurements of insulin resistance or other cytokines apart from a correlation to visfatin (r= 0.26, 0.40, 0.42 & 0.0.44; all $p<0.01$), and hsCRP (r= 0.61, 0.31, 0.24 & 0.04; $p<0.05$ at baseline, 3 & 6 months) at baseline, 3, 6 & 12 months respectively.

Correlations within each dietary group only confirmed a relationship with visfatin for LCD and LGL, but the correlation to hsCRP was not clearly evident. Visfatin will be discussed next in this chapter.

Figure 30: Variation in cytokine levels by percentage

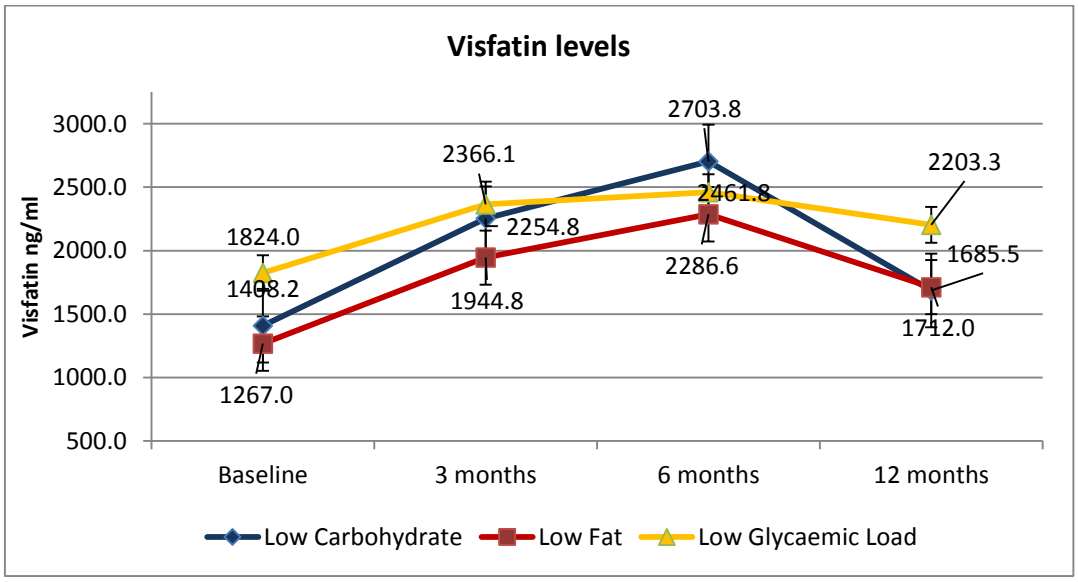


9.4. Changes in Visfatin

Visfatin levels rose in all three groups with levels rising by 60, 92 & 20% in the LCD (all $p<0.05$), 53, 80 & 35% ($p<0.01$ at 6 months) for LFD, and 30, 35 & 20% ($p<0.05$ at 6 months) for LGL.

For all three groups peak visfatin levels were at the 6 months (Figure 31). No between group differences were demonstrated for absolute visfatin levels. The differences in percentage reduction was significant for LCD vs. LFD at 6 & 12 months ($p<0.05$), and for LCD vs. LGL ($p<0.05$) for the same study points.

Figure 31: Visfatin levels throughout the study period



Graph demonstrates the changes in visfatin at each study interval with standard error

As with resistin, visfatin did not display any correlation to anthropometric measurements, markers of insulin resistance, lipid parameters, nor cytokines, with exception to the correlation to visfatin that was previously mentioned, as a study cohort or in individual dietary groups.

The positive correlation to visfatin was noted among the LCD (r= 0.40, 0.41, 0.49, & 0.33; $p<0.01$ at 0, 3, & 6 months) and LGL (r = 0.25, 0.42, 0.45 & 0.60; $p<0.01$ at 3, 6 & 12 months) arms.

9.5. Changes in Retinol Binding Protein-4

Changes in levels of RBP-4 during the study were small with final readings all higher than baseline levels (Table 49 &Figure 30). Changes in the LCD were -14, -7 & 4% ($p<0.05$ at 3 months), at 3, 6 & 12 months respectively, for LFD -6, -5 & 2% and for LGL 3, -6 & 11% at the same study intervals.

No within group differences was observed apart from an initial fall in RBP-4 in the LCD group. Between group differences were only present at the three month stage, between LCD vs. LFD, and LCD vs. LGL (both $p<0.05$).

No correlations were demonstrated for anthropometric measurements, markers of insulin resistance, lipid parameters, nor changes among the other cytokines with RBP-4 for the whole study cohort or the individual study groups.

Table 49: Changes in cytokine levels (RBP-4, PAI-1, hsCRP)

	Low Carbohydrate			Low Fat			Low Glycaemic Load		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Retinol Binding Protein									
Baseline	91.27±42.0	38.6	205.6	94.2±65.7	43.8	421.1	95.4±40.9	32.0	216.0
3 months	75.9±25.3♦	31.3	127.2	88.6±35.2	30.5	186.4	96.6±30.2	45.3	162.4
6 months	88.2±41.3	39.8	199.2	88.9±37.4	0.2	180.3	94.0±46.7	25.0	277.4
12 months	93.3±59.4	44.9	295.9	95.4±38.2	27.0	180.1	98.7±36.7	38.8	191.0
PAI -1(ng/ml)									
Baseline	95.0±60.8	16.4	269.5	63.3±36.4	22.1	175.8	90.1±62.7	8.2	268.2
3 months	66.2±65.2	5.9	252.8	50.8±33.0	4.4	176.4	72.5±57.0	14.4	245.5
6 months	46.3±30.8*	14.8	632.1	49.9±25.9	17.5	110.1	58.3±34.0*	21.0	148.9
12 months	58.0±36.9♦	5.9	252.8	61.9±22.5	19.3	707.7	54.3±43.5*	18.2	227.0
Hs CRP(ng/ml)									
Baseline	5286±4610	862	17839	5118±5504	27	21826	5698±11857	361	75422
3 months	5097±4320	687	16026	4602±6300	203	27680	4231±4943	351	20581
6 months	4675±4463	954	18316	4540±7099	0	30199	4207±4959	200	25808
12 months	3881±4387	534	19401	6553±13219	473	70140	3170±3644	401	17056

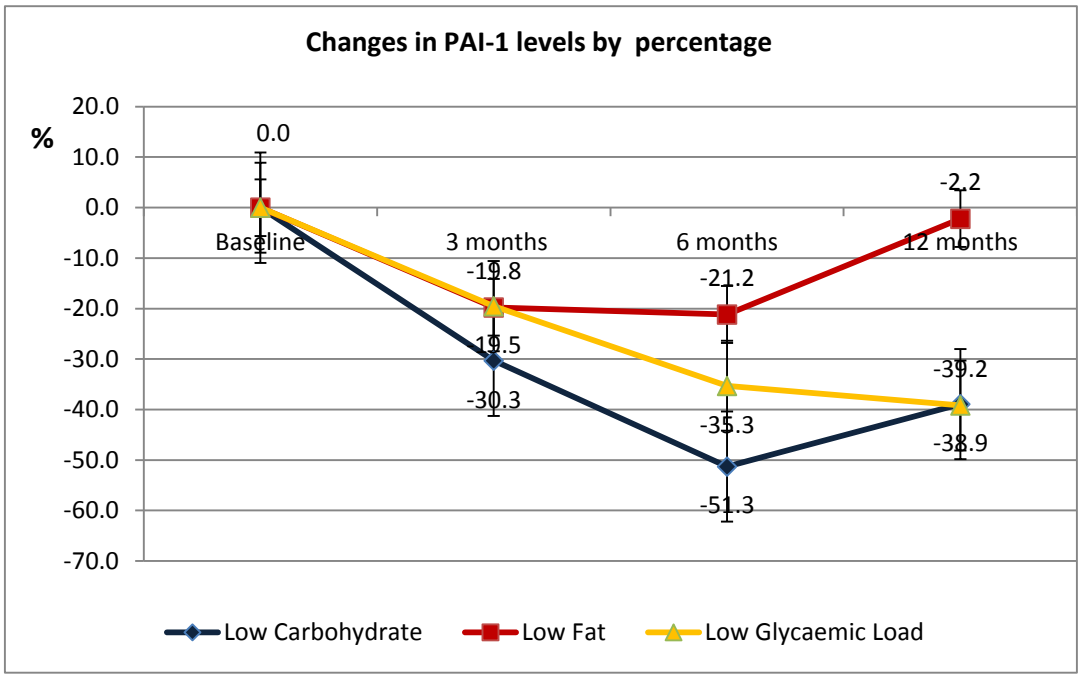
♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to within group changes from baseline)

9.6. Changes in PAI-1

PAI-1 levels fell in all three groups at 3, 6 & 12 months (Table 49 & Figure 33) as individuals lost weight. This was by 30, 51 & 39% ($p<0.05$ at 6 & 12 months) in the LCD, by 20, 21 & 2% (all p=NS) in LFD, and by 20, 35 & 39% ($p< 0.01$ at 6 & 12 months) in the LGL groups. As with the majority of other cytokines peak changes were at 6 months. No differences between the groups were observed at any time point for absolute value or the percentage change.

Despite sustained PAI-1 reductions observed for both LCD and LGL groups, no correlations were demonstrated for weight, BMI or waist measurements, lipid profile, markers of insulin resistance or any of the measured cytokines for the whole study cohort or as individual groups.

Figure 32: Percentage changes in PAI-1 levels



Graph demonstrates PAI-1 levels within each group at each study interval with standard error

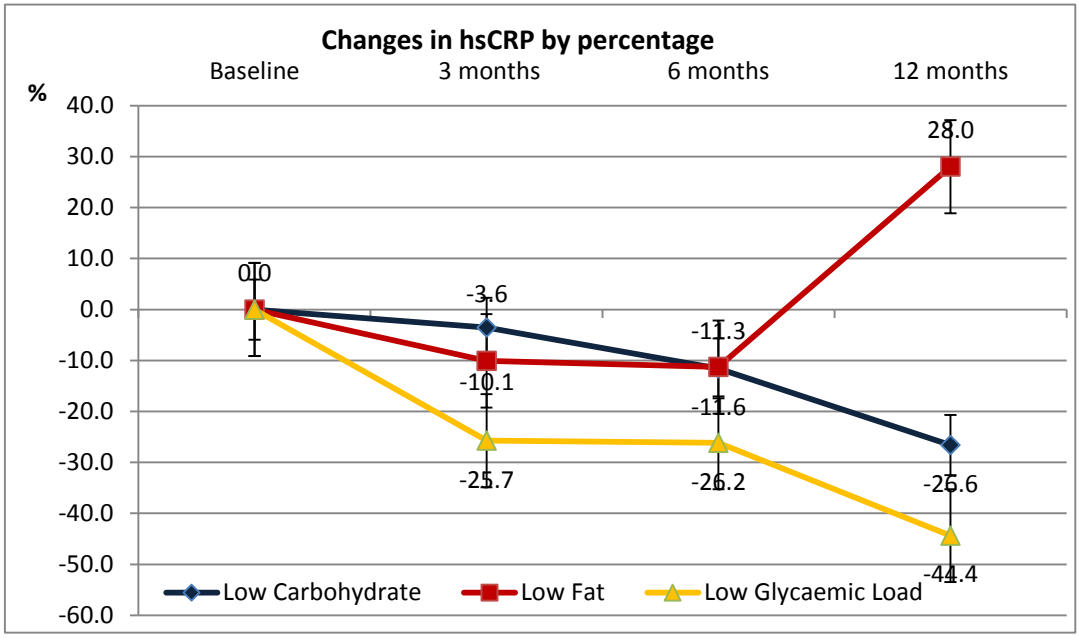
9.7. Changes in hsCRP

Changes in hsCRP levels within the three groups were -4, -11 &-27% (all p=NS) for LCD group, -10, -11 & +28% for LFD (all p=NS), and -26, -26 & -44% (all p=NS) in the LGL at 3, 6 & 12 month respectively (Table 49, Figure 34). None of the changes within or between groups were of statistical significance.

HsCRP levels positively correlated to BMI (r= 0.19, 0.51, 0.35 & .025; p<0.05 at 3, 6 & 12 months), and waist measurement (r=0.34, 0.28, 0.22, 0.24; p<0.05 at 3, 6 & 12 months) for the study cohort at baseline, 3, 6 & 12 months respectively. No correlations were noted for lipid parameters or measurements of insulin resistance.

Within the groups, hsCRP positively correlated to BMI in both LCD (r = 0.47, 0.49, 0.42 & 0.20; p<0.01 at baseline, 3 & 6 months) and LFD (r = 0.39, 0.78, 0.39, & 0.31; p<0.05 at baseline, 3 & 6 months) at 0, 3 & 6 months but not in the LGL group.

Figure 33: Percentage changes in hsCRP



The graph demonstrates hsCRP levels within each group at the study intervals with standard error

Positive correlations of hsCRP with both insulin (r = -0.02, 0.42, 0.62 & 0.59; $p<0.01$ at 3, 6 & 12 months) and with insulin resistance (r = -0.09, 0.44, 0.62 & 0.62; $p<0.01$ at 3, 6 & 12 months) at 3, 6 & 12 months were noted in the LGL group but not for LCD or LFD. These correlations appeared specific to the LGL group and were not demonstrated in the whole cohort.

Similar to the results observed with most of the adipocytokines, correlations between hsCRP and the measured cytokines were not present or not meaningful with many of the correlations occurring through chance and not trend. HsCRP positively correlated with resistin (r= 0.61, 0.31, 0.24 & 0.04; $p<0.05$ at baseline 3 & 6 months), leptin (r=0.32, 0.30, 0.25, & 0.13; $p<0.05$ at baseline 3 & 6 months) when the whole study cohort was analysed. This relation was only demonstrated in leptin among the LGL dietary intervention group (r = 0.33, 0.23, 0.46 & 0.49; $p<0.01$ at 0, 6, & 12 months).

10. CHAPTER 10: Discussion of the Results

10.1. Discussion on changes in Anthropometric Measures and components of the Metabolic Syndrome

It is well recognised that all dietary interventions have poor retention with 12 month concordance rates being low. Most dietary studies report 20- 30% drop-out rates at 6 months[237;393;588] and 30 to 50% at 12[72;133;164;192]. A two year study managed to retain 95% at 1 year and 84% of all participants at 2 years, but the participants all worked within the research faculty, a nuclear research institution, where the clinics were in-house and all food was provided and pre-labelled which would have encouraged compliance and concordance to study diets[482].

When first recruiting for this study the aim was for it to be an intention to treat with all recruited taken into account. Data was intended to be processed in the form of last value carried forward. Most individuals dropped out prior to the second blood sampling stage which was at 3 months and would not even return for a one of fasting blood sample. This would have meant that almost over 20% of the results would have been calculated using a baseline sample only, therefore it was felt best to undergo the analysis using only the completers. There is an appreciation that using the completers only will mean that the study will have been biased in recording the results of only the more compliant individuals or those who had been satisfied with their weight loss. As the data contained individuals who had effectively loss weight as well as those who had put on weight throughout the year study period then it potentially excludes that bias. There has been criticism of intention to treat analysis, as the data can be potentially diluted by results of those who have dropped out, and the interpretation of results will be affected and potentially misguided depending on the heterogeneity seen in those who have dropped out or were non-compliant. It is actually advised that intention to treat analysis be undertaken when all efforts have been made to minimise missing data to reduce heterogeneity in results[203;235].

10.2. Weight and waist

Most of the dietary studies comparing high-fat low-carbohydrate, or Mediterranean-like diets were of a 6 to 12 month duration involving between 20 to 100 subjects. Average weight loss within these trials ranged between 2-10kg weight loss in the low carbohydrate groups, 2-6kg in the low fat groups and 3-5kg in the low glycaemic index groups[132;164;343;393;466;588]. Weight loss within our study followed a similar pattern to those of the other studies. In fact when compared to the Dietary Intervention Randomized Controlled Trial (DIRECT) which randomised individuals to very similar dietary interventions the results here appear to be favourable [482].

Weight loss in the three study groups reached a maximum trough at 6 months then stabilized following patterns seen in diet trials which reported up to a year or longer. **Figure 34** is the weights of all the participants in the study comparing the weights at baseline, 6 and 12 months. A reasonable separation between the Baseline and the 6 month line intervals are seen for the majority, the period where weight loss is at its peak. On the other hand considerable overlap is noted between the 6 and 12 month lines which is the maintenance phase where most weight losers had attained their maximum weight loss and had slowed down or stabilized.

Figure 34: Combined weight changes for all participants by group

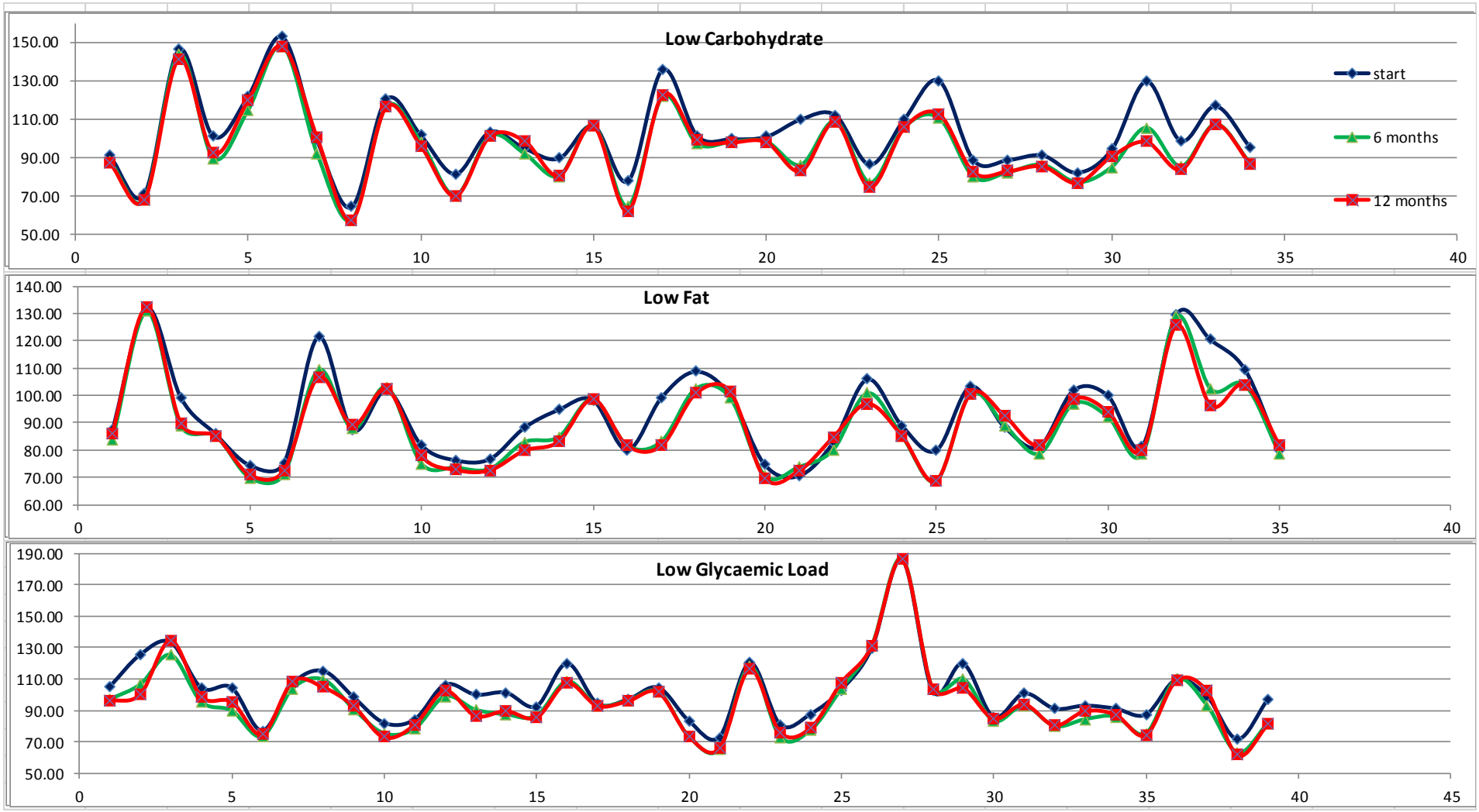
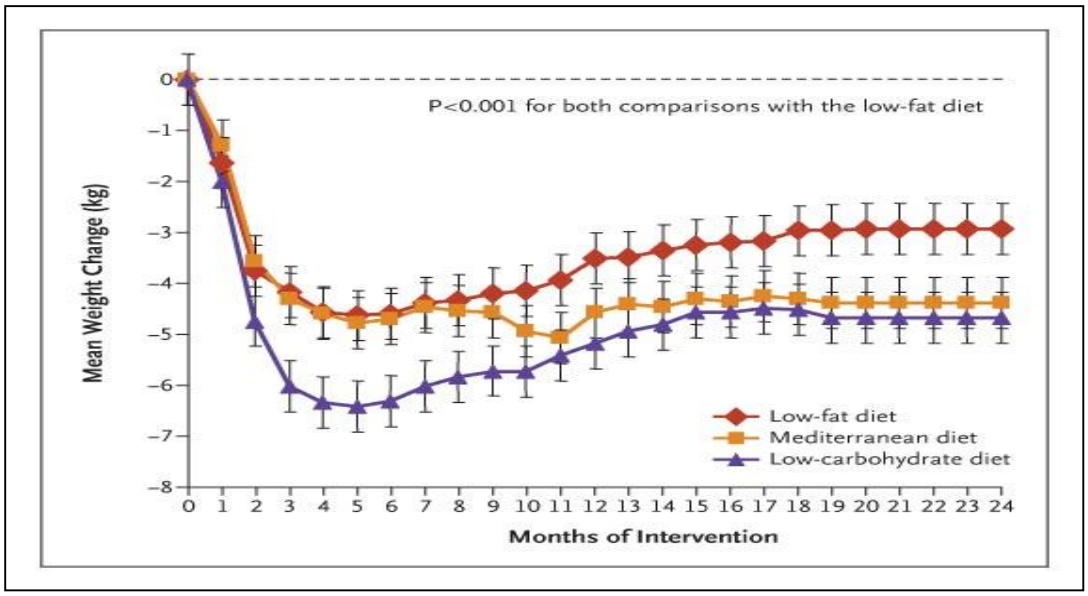


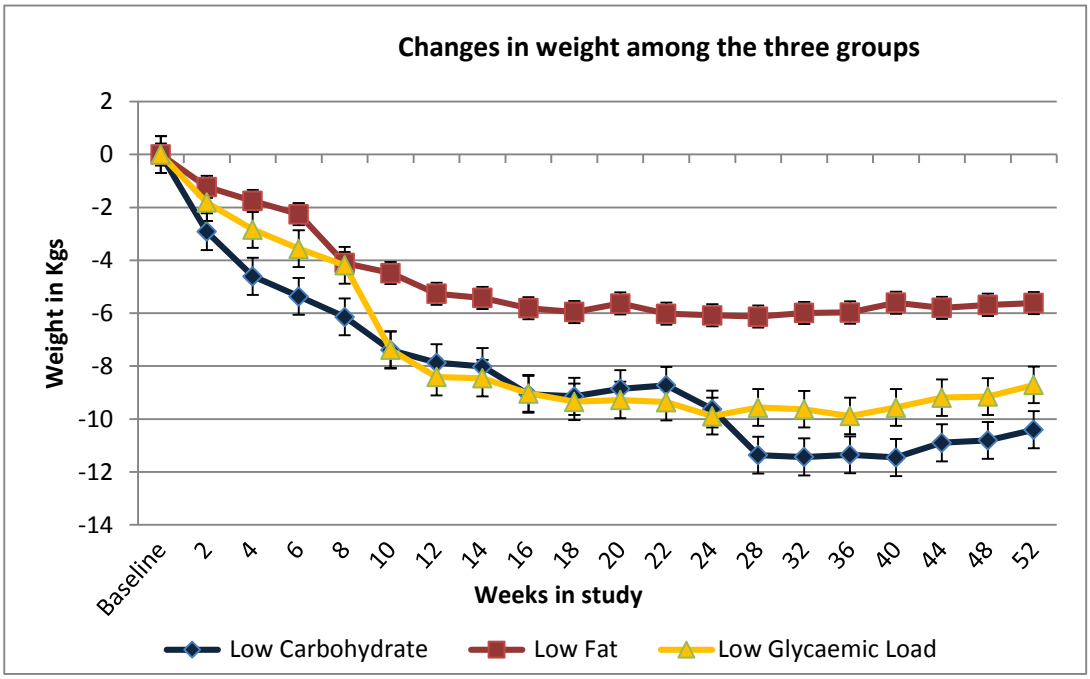
Figure 35: Weight changes over 2 years in the DIRECT study



Reproduced with permission from Shai et al 2008[482], Copyright Massachusetts Medical Society.

In the DIRECT study, the three study arms were starting to rise at 12 months, but the curve remained flat with subjects appearing to maintain weight for up to 24 months (Figure 36). Recently the 4 year follow up data were published which revealed that the control low fat group had regained 78%, the low carbohydrate 64%, and the Mediterranean 30% of the weight lost [476].

Figure 36: Changes in weight among the three study groups



Similar trends were seen among the subjects studied in this study (Figure 37), but as it did not extend beyond 12 months it is difficult to speculate on whether weight would have remained stable or returned to baseline. A letter was sent to all subjects a year after completion asking for details of weight but very few replied. The results at least imply that LCD or LGL methods are superior at achieving initial weight loss and maintaining it for a period of time. The LFD group were the group with the lowest weight at baseline which may account for them losing less kilogram weight as compared to the other groups, but even the percentage weight loss was the least among that group. To demonstrate this a multi-centre European trial randomised 700 individuals after successful weight loss in an initial calorie restriction phase to one of 5 arms: low protein and low glycaemic index, low protein and high glycaemic index, high protein and high glycaemic index, high protein and low glycaemic index, or control (standard regulations by country) for a 26 week maintenance phase. All arms had regained their weight loss apart from the high protein and low glycaemic index group [307].

Weight loss is very much an individual thing and Figure 29 clearly demonstrates that there were individuals who did better than others while some failed to show any weight reductions despite regular counselling and adjustments to caloric intake as befitted their weight and degree of activity. It would be unfair to assume that the lack of response was purely due to lack of compliance as activity levels and basal metabolism are other factors that contribute. In this study it was decided not to recommend any lifestyle changes in the form of exercise to avoid confounding. It was observed that many of the successful participants admitted to increasing their level of exercise as they lost weight and began feeling lighter and healthier. Unfortunately our data did not identify whether a particular group had adopted exercise more than the others.

Waist circumference is considered a marker of visceral adiposity and as such is one of the components of metabolic syndrome. Reductions in waist circumference, as such can be a better predictor of CV risk improvement than weight. Studies have confirmed the correlation between weight, waist, BMI and visceral adiposity and implied that a 3kg weight loss can be equivalent to a 3cm waist circumference reduction in men and women[371;372]. Certainly changes documented in this study were

consistent with these findings, with waist reductions in LCD and LGL averaging 1cm per kg, and LFD 2cm per kg weight loss. The superior numbers may imply that LFD is therefore a better diet for reduction in visceral adiposity, however this will be difficult confirm as waist measurement failed to correlate to any markers of visceral adiposity or insulin resistance in this group in particular.

10.3. Components of the Metabolic Syndrome

The data clearly identifies that the dietary interventions appear to be successful in reversing the components of metabolic syndrome.

10.4. Blood Pressure

Blood pressure is a variable measurement that can be affected by several factors including stress, be it physical or emotional. Attempts at reducing white coat hypertension and anxiety were addressed in this study by allowing the subject to rest calmly, and supine for five minutes prior to measurements being taken.

Individuals studied on the Dietary Approaches to Stop Hypertension (DASH) diet sustained 5.5 and 3mmHg reductions in systolic and diastolic readings. These reductions were up to 8.5mmHg systolic in those who achieved an 8kg weight loss equal to reports from a meta-analysis of randomised controlled trials, by Neter el al, which suggested that 1kg weight loss equated to a 1mmHg reduction in systolic blood pressure. Greater reductions were achieved by the addition of exercise and in those already on anti-hypertensive therapy[41;44;387]. Diastolic readings did not display any form of predictable pattern. Blood pressure reductions among the LCD and LGL groups were similar to the numbers reported in the meta-analysis and appear to be better than those reported by Nordmann. In a meta-analysis examining the effect of low carbohydrate diets on cardiovascular risk he suggested that although low carbohydrate diets may have an initial beneficial effect on systolic blood pressure, this was not sustained long term. Blood pressure changes in all but one of the trials included in the meta-analysis were small (2.4/1.8mmHg) and the trial durations varied between 6 and 12 months. Similarly, results for DIRECT demonstrated no difference in blood pressure reduction across their three groups[393;482]

It was of interest to note the changes in anti-hypertensive therapy during the study period. To limit difficulty in interpretation individuals who had required

alterations in antihypertensive therapy up to three months prior to the study had been excluded. The alterations certainly do affect how the blood pressure data are to be interpreted but the positive factor to be established is that approximately 20% of individuals were able to discontinue blood pressure medications through dietary intervention. These discontinuations were particularly noted among those allocated too an LCD or LGL dietary life-style but not the conventional, low fat recommendations. Not only did this group show the least reductions in both systolic and diastolic blood pressure, it was the group which had the most increases in numbers of anti-hypertensive therapy and the least likely to discontinue. This implies that, assuming external factors remained unaffected, a LFD may be deleterious or unsuitable for individuals known to be hypertensive.

Why did the LFD group do worse? Were there any dietary causes for this? Could they have a higher salt intake in comparison to the other two groups? Unfortunately the analysis from the food diaries does not suggest this. Salt intake went down among all three groups at the start of the study and for the LFD group in particular was persistently and significantly lower than in the LCD or LGL groups. This is discussed further in the dietary analysis section.

10.5. Lipids

The rise in total cholesterol seen within the LCD group was not unexpected as it is believed to be partly attributable to the increase in LDL-cholesterol which could have resulted from the increased intake of saturated fat if individuals were following the prescribed dietary recommendations. Of note, the rise in both total and LDL-cholesterol was not statistically significant, and at its maximum was 6% for total cholesterol and 8% for LDL-cholesterol. Some studies have reported that although LDL-cholesterol rises in low carbohydrate diets, cardiovascular risk may not be significantly affected as the particle sizes were larger and more buoyant and thus believed to be less atherogenic[485]. The reduction in LDL-cholesterol was only significantly demonstrated in the LGL group.

Studies comparing high and low glycaemic index diets have shown that lowering the glycaemic load of carbohydrates can lower LDL-cholesterol regardless of the degree of weight loss[452;500]. Despite the actual total fat intake being the lowest among the three groups, by at least 30%, LFD had no effect on total or LDL-cholesterol. Similar increases in total and LDL-cholesterol among low carbohydrate diets were reported in other dietary trials [164;393;467]. Many studies have demonstrated favourable changes in total and LDL-cholesterol in individuals placed on low glycaemic index or load regimens[129;259]. The evidence has been inconsistent in that some have reported low glycaemic regimens to be superior[258;482] and others equal[139;229] to low fat regimens in improving LDL-cholesterol.

Do these changes in LDL-cholesterol signify much? The report from the Cholesterol Treatment Trialists' (CTT) meta-analyses indicates that for every 1mmol/l reduction in LDL-cholesterol there is a 12% reduction in CV events with an additional 25% reduction for each subsequent year[96]. If so then CV risk in the LGL arm will have been reduced by 4% and in the LCD arm increased by 3%. This assumes that the CTT data, largely from statin trials, apply to LDL-cholesterol changes achieved through dietary modification.

Cardiovascular outcome studies have all emphasised the importance of LDL-cholesterol lowering [101;481]. In this study LDL-cholesterol reductions were not successfully achieved in any of the dietary interventions apart from the LGL group which did display a modest reduction of 4.8% (0.3mmol/l). These results are similar to other dietary studies where LDL-cholesterol levels varied little through dieting with or without pharmokinetic agents, but appeared to be influenced by the incorporation of physical exertion [142;227;426;573;574].

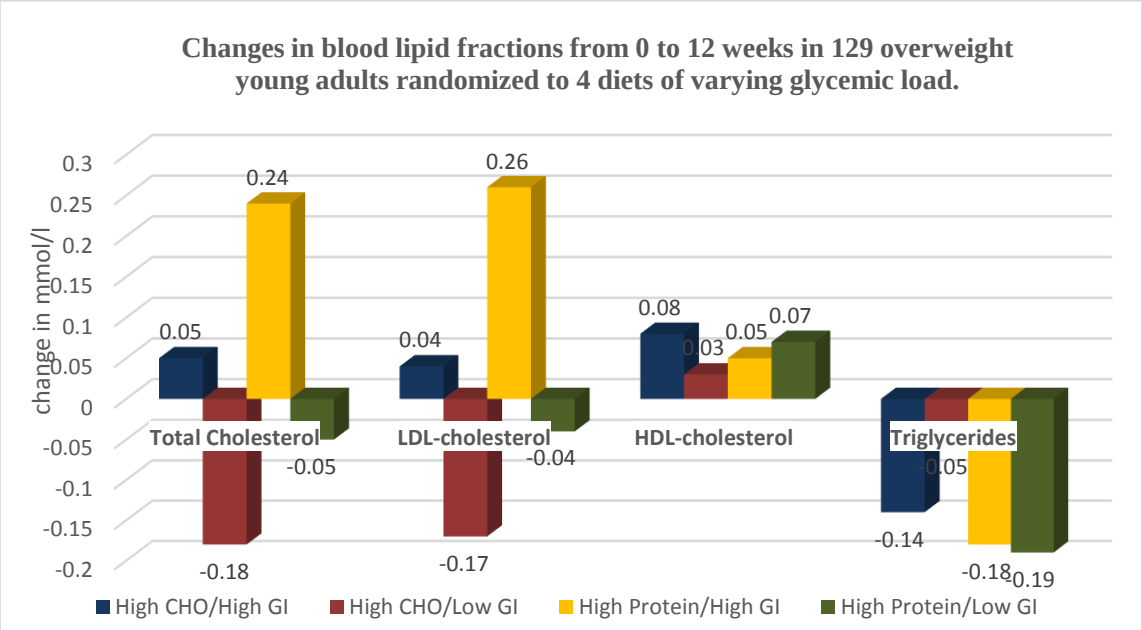
Dietary studies using low fat, low carbohydrate or a low glycaemic load approach have in majority reported positive improvements in HDL-cholesterol and triglyceride levels in all the dietary interventions [108;274;393].There is a lot of speculation on how important a role does HDL-cholesterol play in improving CV risk. Is the rise seen within the three study arms of any real meaningful clinical value?

The earlier trials looking into addressing HDL-cholesterol and triglyceride levels had initially shown some improvement in cardiovascular risk and mortality with the use of fibrates [453]. The popularity of fibrates lessened with the introduction of statins and the focus of management shifted to the lowering of LDL-cholesterol which was now achievable with these newer agents. The more recent randomised controlled trials have failed to show a significant benefit in improving cardiovascular risk outcomes with fibrates [278;525]. Critics have suggested that the lack of results is possibly due to the fact that most individuals with elevated CV risk will already be on a statin and trials looking at lipid-lowering in naïve subjects with high risk will be ethically difficult to support. Furthermore, unlike earlier trials most of the recent fibrate studies have failed to demonstrate the expected changes in triglycerides and HDL-cholesterol that is expected from fibrates[278;525]. Alternative pathways for regulating the lipid profile by either raising HDL cholesterol [53], or lowering triglycerides [141] are currently being considered but success has been poor with many studies discontinued due to adverse events. What is important is the functional integrity of HDL particles rather than just HDL-cholesterol concentration. How HDL-cholesterol is raised is relevant, although one might consider that dietary manipulation may be physiological. In the face of current evidence, it is difficult to know whether the improvements seen in HDL-cholesterol and triglycerides were enough to counteract a potentially deleterious rise in LDL cholesterol.

Certainly a LGL diet appears to tick all the boxes for improvements in lipid profile, but is it enough to rule out an LCD diet due to its effect on LDL-cholesterol levels? Perhaps any diet that successfully helps an individual to lose a significant amount of weight may provide benefits that outweigh these possible disadvantages.

The lipid changes seen within the study were similarly demonstrated in a trial studying the effect of differing glycaemic loads on cardiometabolic factors. The first two diets were both high in carbohydrate with one consisting of high glycaemic index food (High CHO/High GI) and the other low glycaemic index (High CHO/Low GI). The second two diets were high in protein but again varied in the quality of glycaemic index carbohydrate (High Protein/High GI and High Protein /Low GI). Individuals were studied for 12 weeks (Figure 38).

Figure 37: Changes in lipid profiles in diets with varying glycaemic load

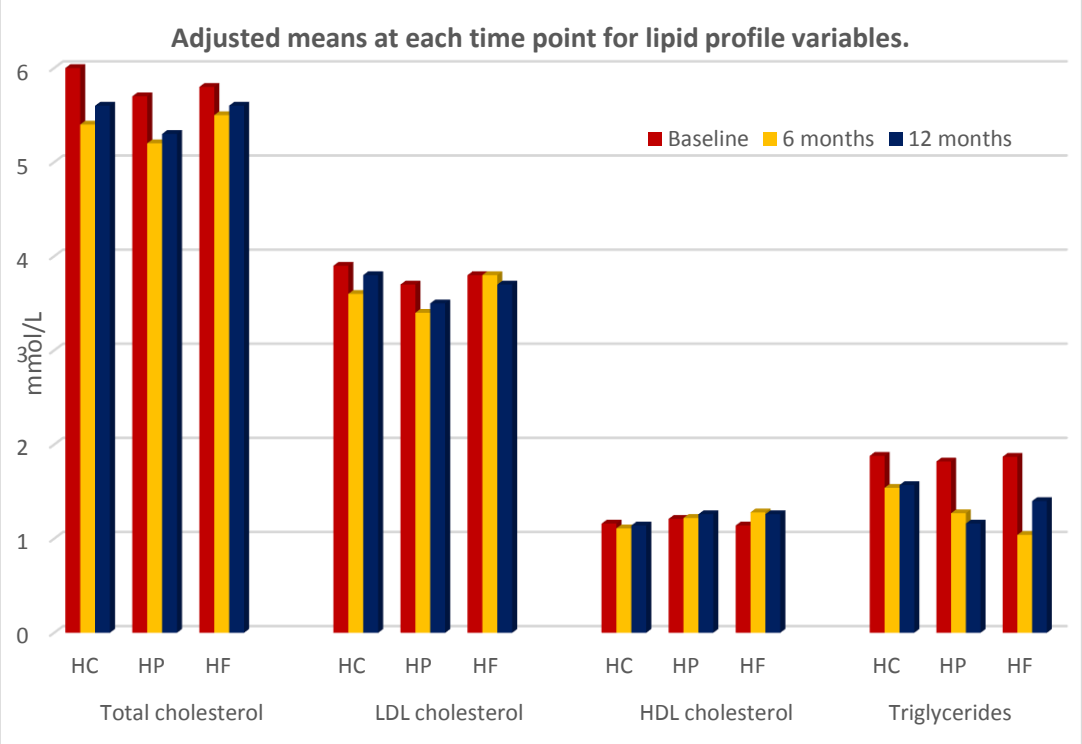


Reproduced with permission from Brand Miller et al, 2006 [362]

Reductions in total and LDL-cholesterol were only present in the low GI arms, and particularly noted in the high CHO but low GI group. Increases in both these factors were evident in both high GI groups with the largest increase being in the high protein, high GI group. HDL-cholesterol universally rose in all the arms and triglycerides fell secondary to the dietary effect[362].

Mann *et al* in their trial of 93 obese females on a high carbohydrate (HC), high protein (HP) or high fat (HF) diet, followed for 12 months demonstrated that Total cholesterol fell in all three groups but to a lesser degree in the high fat arm. LDL-cholesterol fell in the high carbohydrate and high protein groups but remained stable in the high fat arm. Triglycerides levels fell and HDL-cholesterol rose in all three groups with patterns once more similar to what was seen in this study[359].

Figure 38; Long-term effects of popular dietary approaches on lipid profile



Adapted from Long-term effects of popular dietary approaches on weight loss and features of insulin resistance[359]
HC: high carbohydrate, HP: high protein, HF: high fat

One area we have not explored in this research is analysing lipoprotein sub-fractions which will assess the quantity and quality of both HDL and LDL particle size. Do any of the diets have an effect on altering the particles from small, dense atherogenic ones, to less quantities of larger less harmful forms[352]?

10.6. Cardiovascular Risk Reductions

If the current lipid and cardiovascular protection guidelines are to be strictly followed, as in therapy for primary prevention, then only the LFD group would have qualified for therapy at the end of the study[382]. Admittedly both the LFD and LGL groups had started with a higher baseline 10 year CV risk calculation as compared to the LCD group. In spite of this, both LGL and LCD achieved calculated risk reductions of up to 18 and 19% respectively which were sustained up to the end of the study, while maximum reductions in the LFD arm were only 9%.

Dietary trials have all commented on cardiovascular risk by analysing each individual risk factor but none appear to have reported on calculated CVD risk reductions. Primary prevention statin trials have all reported reductions in CV risk and mortality of 20-30% which heralded their use in adults almost empirically[100;481;489]. A recent meta-analysis on statin lipid lowering reported similar results while commenting that the benefits were not sustained and recommended that subjects be assessed on an individual basis for statin therapy[440].

If dietary interventions are first line measures for cardiovascular risk prevention then certainly it makes sense to ensure that the advice provided helps improve outcomes. The Look AHEAD trial was terminated early by the National Institutes for Health. This was a multi-centre randomised controlled trial looking at cardiovascular outcomes in 5,000 individuals with type 2 diabetes randomised to intensive lifestyle intervention or standard. Other care was provided by the participants' regular medical doctors. The study was intended to run for 13 years but was stopped at 11 years as no improvement in cardiovascular risk in favour of the interventional arm was demonstrated, despite an initial weight loss of 10% that was maintained at 5%, and significant improvements in other parameters of cardiovascular risk[57;255;444;547].

In a longitudinal review of mortality data from 6,000 survivors of the third NHANES (individuals who were known to be healthy), a 15% weight loss was found to be associated with an increased risk of all-cause mortality in this reportedly healthy

cohort. The increased risk was for non-CV disease among obese men and all-cause mortality, favouring CV disease, for overweight females[250]. The researchers admitted to a number of areas of weakness within the study including not recording whether the weight loss was intentional or not, inability to follow did some lose more weight and regain it, was maximum body weight initially recorded. They had tried to exclude underlying sinister disease by excluding all who had passed away within three years of recruitment.

Both LGL and LCD lowered CVD and CHD risk factors by acceptable margins in spite of the increase in total and LDL-cholesterol demonstrated in the LCD group. Look AHEAD has suggested that dieting and healthy lifestyle interventions alone may not be enough to reduce cardiovascular outcomes but can manipulating dietary macronutrients be of benefit? If we are to take CV risk as a whole rather than independently addressing each individual component than surely we should be moving away from low fat, refined carbohydrate-loaded regimens which display modest changes in general but as a whole do not appear to provide any apparent significant protective benefit.

10.7. Discussion of changes in nutrients (dietary analysis)

Dietary analysis is rarely an absolute representative of actual dietary intake unless the food consumed had been prepared and provided by the researchers so the exact weights and macro-nutrients could be identified. Even when subjects are absolutely meticulous with recording their entire dietary intake within the required time, there tends to be a subconscious reduction in food intake on the monitoring days. This is partly due to conscious good behaviour and the need to comply on paper and secondly, to reduce the inconvenience caused by the weighing and measuring process. Although the researchers emphasised the importance of the diaries being complete the results will have to be viewed cautiously. Dietary trials which have reported on macronutrient intake have used food diaries to check that dietary recommendations were achieved and there was compliance[295;307;462]. The variations achieved in the macronutrients in our study for the LCD and LFD were similar to those reported in a study from New Zealand who looked at individuals on Atkins, the Zone Diet and standard recommendations for a period of 6 months[358].

Although participants randomised to the LCD group were permitted unrestricted calories, they actually dropped their intake by approximately one fifth of their pre-study average daily caloric intake. This reduction was equivalent to the reduction seen in the LFD group and more than that of the LGL group both of whom had been prescribed specific energy intakes calculated accommodate to weight, gender and level of daily activity. Atkins had professed in his book that the ketosis associated with the low carbohydrate intake results in appetite suppression within 48 hours [40]. It is reasonable to assume ketosis may be partially responsible as there had been reductions in carbohydrate intake so that it made up only 10% of the daily calorie intake. The average carbohydrate intake at both 6 weeks and 3 months was approximately 40gm, a fifth of what they had been partaking at baseline and in keeping with the initial 20gm restriction followed by a gradual 5gm/week incremental rise.

More likely the cause would be the increase in protein intake. Daily protein intake in both the LCD and LGL groups increased by approximately 12%. Higher increases had been expected for the LCD group as the assumption was that the carbohydrates would have been substituted with protein products. Although there was an increase in protein intake this was surpassed by the 25% increase in fat intake. Studies in the past have shown that an increase in protein intake from 15 to 30% of the daily food allowance was associated with appetite suppression irrespective of the carbohydrate intake[267;565]. These reports were further supported by a systematic review (of 50 articles) of high protein diets which concluded that high protein diets did have a positive effect on satiety, thermogenesis, body fat loss and energy expenditure[212;312]. Other studies have implied that the positive effect high protein intake had on satiety was unsustained for long periods[335].

Carbohydrate intake is expected to be the largest single source of energy intake in today's diet with current recommendations pronouncing that it should make up 45-60% of daily caloric intake[6;159;526]. The National Diet and Nutrition Survey published that the average carbohydrate intake in individuals aged 19-65 years was 227gm/day comprising 47.7% of total energy intake[123]. Our cohort of study subjects had similar amounts of carbohydrate intake (211, 228 and 237gms/day for LCD, LFD and LGL respectively), which was approximately 40% of the total daily intake, a level which is below the current national dietary recommendations. Of note carbohydrate intake in both LFD and LGL fell to even lower levels and remained so till the study end. Is this a true reflection of the cohort? Of course there is the possibility that certain items (e.g. snacks or drinks) containing hidden carbohydrate were undocumented.

To discuss the carbohydrate intake in the LCD group, the recommendations as per the Atkins' book are to restrict carbohydrate intake to less than 20gms per day during the induction phase which could last 2 weeks or as long as the candidate preferred. Once satisfied with their initial weight loss, individuals would then move on to the next phase "on-going weight loss" followed by "pre-maintenance" during which carbohydrate intake is gradually increased by increments of 5gms per week until a point at which loss has stabilized. If we assume that most undertook a two week induction phase then 40gm/day of carbohydrate at 6 weeks is on target for the LCD group. In his book Dr Atkins recommends that the carbohydrates introduced

should include salads, fresh cheese, seeds, berries legumes and whole grains, all low glycaemic load items[40]. From the dietary analysis data 50% of the LCD group's carbohydrate intake at all the study intervals with exception of the start was refined carbohydrate. Many arguments could justify the reason for the relatively high percentage of refined carbohydrate. First, being the lack of knowledge or awareness of the quality or quantity of carbohydrates included, despite education being supplied when interim food diaries were reviewed at each visit. A second cause is a craving for sweet things as a snack which many of the participants reported and were keen to gratify as soon as they were allowed any carbohydrate increase. Third is that busy lifestyles mean that pre-preparing meals is not usually convenient or desirable. Easy-to-obtain snacks will usually contain refined sugars. If the snack is a fruit which is easily obtainable (i.e. apple, banana or pear) these tend to be more carbohydrate-laden in comparison to melon or berries. Fourthly, financial restrictions can play an important role as most items of a low glycaemic load tend to be dearer.

Total fat intake reductions among the LFD were expected on the basis that this was a low fat diet. Reductions were seen in all types of fat intake with no change in the proportion of the different components. Individuals were instructed to choose low fat options but had no restrictions on the type of fat chosen which may account for the lack of variation. The LGL group had been counselled to specifically alter their fat intake by choosing monounsaturated fat rich products e.g. olive oil spreads, rapeseed oil or olive oil and avoiding polyunsaturated fats e.g. low fat spreads and standard vegetable oils. Despite active advice to alter the type of fat intake there was only a small reduction in saturated fats (26.8gms/day to 21.2 & 20.7mgs/day at 3 and 6 months $P<0.05$) which was not sustained to 12 months, and no changes in the amount or proportions of monounsaturated and polyunsaturated fats. Despite this lack of variation the more positive lipid effects were noted within the LGL group confirming that the quality and quantity of fat intake was not the major denominator in an individual's lipid profile. Such suggestions had previously been made in a study of young children where dietary intake varied in quantity and quality of fat intake. A correlation was noted between the caloric amount of carbohydrate intake and aspects of the lipid profile but none with the type of fat ingested [297]. In a meta-analysis of 14 studies comparing how monounsaturated or polyunsaturated fats affected lipid profiles, no significant changes were noted for total-cholesterol, LDL-cholesterol, or

HDL-cholesterol. Polyunsaturated fat were reported to have a superior lowering effect on triglycerides[183]. These changes were not observed in our study but then it could be argued that although the intended fat intake was supposed to differ between the diets, the actual changes were small. Additionally the present study did show that the LGL (higher monounsaturated fat) group had a more positive impact on total-cholesterol, and HDL-cholesterol.

In the LCD group the increase in fat intake was mainly noted in saturated and monounsaturated fats which rose by 30 and 32% respectively at 6 weeks from baseline levels. The saturated fat intake could potentially account for the rise seen in total and LDL-cholesterol levels. Many studies have confirmed that substituting saturated fats with monounsaturated or polyunsaturated fats helped improve CV risk factors, one of which is the lipid profile [518;558]. In a study looking at high fat ketogenic diets in children with epilepsy changing the fat intake from saturated fats to polyunsaturated oils helped normalise the lipid profile within the seven month study period[150]. Therefore, should an individual want to follow a low carbohydrate diet in the future, they would be best advised to avoid foods high in saturated fat, and choose those options which have a higher proportion of monounsaturated or polyunsaturated fats.

When examining the changes noted in blood pressure there was an expectation that dietary salt intake might have partially accounted for the variations. In fact all groups demonstrated similar reductions in salt intake at the start and this appeared to be maintained for the LFD group, which is the group with the least blood pressure changes, while levels of salt intake rose back to baseline for the LCD and LGL groups, where reductions in systolic readings were up to 5% (8.5mmHg). In addition, the LFD group required more up-titration of antihypertensive therapy than the other groups. A meta-analysis looking at the effect of salt intake on blood pressure suggested that a reduction of six grams of salt per day for four weeks would lower readings by 7/4mmHg for hypertensives and 3.5/1.5mmHg for normotensive individuals [228]. Similar results were noted in the Exercise and Nutrition interventions for Cardiovascular health (ENCORE) study which also documented the superiority of a combined exercise, weight loss and salt restriction regimen to any of the interventions individually [64]. Our study was not geared to look into salt intake or

exercise, so it is difficult to assess how much of an impact either of these might have had on blood pressure.

Reduction in calcium intake was expected in the LCD group where restrictions in carbohydrate intake meant cutting out dairy products including cheese until the induction phase was over. Daily recommendations for calcium intake are 1000 – 1200grams. None of the groups were achieving this at the start and at 3 months the LCD group were only taking 50% of the recommended daily allowance. The only group which appear to maintain calcium levels at recommended levels was the LGL group which implies that those commencing on diets which potentially restrict dairy intake (both LCD and LFD) might need to consider taking supplementary calcium to ensure adequate maintenance.

10.8. Discussion of changes in Insulin Resistance

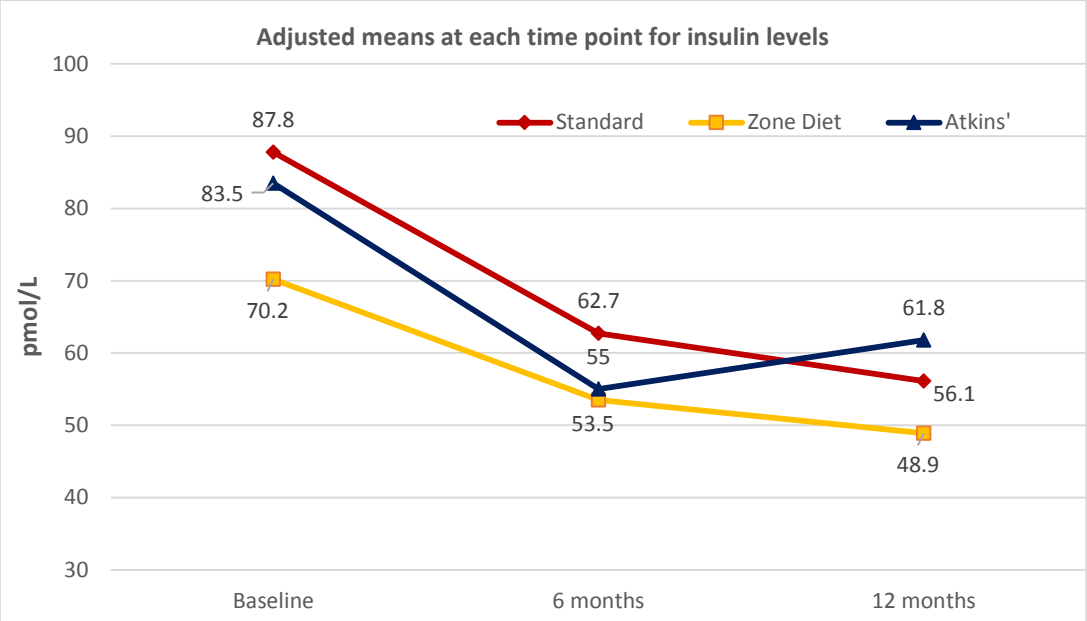
10.8.1. Fasting Glucose and Insulin

The majority of the dietary studies have reported improvements in fasting and post prandial (2 hour) glucose particularly those advocating a low carbohydrate or low glycaemic intake[516;568;569;576]. The glucose lowering effect in these studies appears to be attributed to the caloric intake reduction and weight loss with no particular diet showing a sustained significant superiority. Nordmann's meta-analysis favoured the low carbohydrate groups reporting that fasting glucose levels were lower in this group at 6 months, but the advantage was not present at 12 months [393]. Gannon in some short studies compared high protein to high carbohydrate intake and reported that fasting glucose levels were reduced by 36% in the high protein groups[177;178]. When studying glucose responses in obese females, Layman reported a superior reduction in fasting glucose in the high carbohydrate arm[313], findings that were replicated in another study comparing a high protein to high carbohydrate intake. Fasting glucose levels and glycated haemoglobin in the high carbohydrate group fell by 1.5mmol (18%) and 1.3% (15%) while they were unchanged in the high protein group[469]. Layman's theory for these results was that in order to achieve and maintain fasting glucose lowering it was not enough to reduce carbohydrate intake, but to achieve a balance between gluconeogenesis and the peripheral utilization of glucose without encountering insulin resistance. In our study, only LFD and LGL were statistically effective in reducing fasting glucose. In a Canadian trial, Carbohydrates in Diabetes (CCD), 3 month fasting glucose was lower in the low carbohydrate and low glycaemic index arms but rose with the high glycaemic index group having the greatest effect at the end of the one year trial. Post prandial glucose levels were initially lowest in the low carbohydrate groups, but escaped rising to above baseline. Only the low glycaemic index arm maintained post prandial levels[576].

Although reductions in post-prandial glucose were present and sustained in both the LGL and LCD arms in our study, the differences when compared to the LFD

set were never significant. Reductions in glucose for the LCD group is likely to be linked to the decreased carbohydrate intake and hence glucose utilisation and the subsequent increase in fat metabolism to compensate for intracellular energy requirements. For the individuals on LGL, the lower and slower deliverance of glucose from their ingested meals potentially allows for more efficient disposal and utilisation glucose without insulin over-stimulation.

Figure 39: Long-term effects of popular dietary approaches on insulin levels



Adapted from McAuley et al[359]

A substantial and significant lowering of baseline insulin was observed in all three of the study arms but none appeared to be superior which is similar to the New Zealand study where 93 obese insulin resistant females were placed on Atkins' (high fat), the Zone Diet (high protein) or standard advice for 6 months. Improvements in insulin levels were noted in all groups but superior in the high fat and high protein arms[358]. An extension of the study confirmed the improvements to be sustained in the high protein and standard arms whilst insulin levels, and post prandial glucose rose in the high fat group [359] (Figure 39), whereas in our cohort of subjects the improvement in insulin levels was sustained for the three groups. Admittedly the analysis was based on the completers within the study and potentially those who were most concordant, but in a puritanical way these results can clearly define the outcomes of the individuals who persevered with the dietary allocation.

Of note not only were the insulin reductions sustained in those on LCD, this was the only group where insulin levels correlated to weight and waist measurements in keeping with the whole cohort analysis. LGL had correlated insulin levels to waist measurements but this relationship was not identified in the LFD group Was the lack of response due to the degree of weight loss not being enough to produce any meaningful effects. It was the only group that did not achieve the overall 5% recommended target weight loss, managing to reach 4.4% in comparison to 6.9% (LGL) and 8.8% (LCD) or is there another mechanism.

10.8.2. Insulin Resistance

Reductions in insulin resistance followed similar patterns noted in other dietary intervention trials. Certainly in the meta-analysis looking at the Mediterranean diet, it was felt that all individuals who complied with the principles of a low glycaemic load intake showed more positive improvements to insulin resistance as compared to those with poor compliance [274]. The meta-analysis of low glycaemic index diets from Brand-Miller did not assess insulin resistance but glycaemic control in individuals with Type 2 diabetes, and reported that low glycaemic index diets were superior to high glycaemic index diets, reducing HbA1c by an additional 0.43%. Analysis from the Nurses' Health Survey had reported that a high glycaemic load and low fibre intake were separately associated with an increased risk of type 2 diabetes and when combined the accumulative risk increased 2.5 times[464]. Similar results were reported among the 43, 000 males studied in the Health Professionals Follow up Study[463].

Contradicting this was the Insulin Resistance Atherosclerosis Study which included approximately 1000 individuals with normal or impaired glucose tolerance, and studied the effect that glycaemic index, glycaemic load, and fibre had on insulin secretion, insulin resistance and weight. It concluded that no relationship existed between carbohydrate and glycaemic load or glycaemic index in relation to markers of insulin resistance and that any such findings were coincidental[326]. The researchers

did find an association with increased insulin resistance in those with a reduced fibre intake but in general felt that it was the carbohydrate/caloric load rather than quality that really impacted on these markers through their effect on adiposity.

The relationship between protein intake and insulin resistance has been controversial. Studies looking at amino acid infusions have linked high protein intake to insulin resistance [179;180;310;539]. The European Prospective Investigation into Cancer and Nutrition (EPIC)-NL was a multi-centre prospective cohort study which aimed to study the aetiology of chronic disease. Recently it examined the association between dietary protein and diabetes and reported that animal protein, not vegetable, was associated with an increased risk of diabetes, and increasing dietary animal protein by 5% at the expense of carbohydrate would increase the risk for type 2 diabetes by 30%[501].

On the other hand many dietary interventions have identified the benefit of a high protein, low carbohydrate diet, at least as a short term option for weight loss, improving insulin resistance and other cardiometabolic benefits[129;177;178;561].

Certainly, from the data available in this study, improvements in insulin resistance was sustained in all the study arms although it does not clarify what parameter has the most influence. Weight appeared to only correlate to LCD, and waist measurements to LCD and LGL. Could the improvements be due to weight loss and reductions in visceral adiposity which influence insulin resistance?

To check whether the correlations were related to weight loss, we looked at the 24 individuals who had achieved a greater than 10% body weight loss (9 from LCD, 4 LFD, & 11 LGL). Insulin levels failed to correlate to weight or waist measurements but positively correlated to BMI (r= 0.54, 0.47, 0.45 & .50; all $p<0.05$) in this cohort. Similar patterns were demonstrated for insulin resistance, insulin sensitivity and ß-cell function.

We also looked at the 38 who failed to achieve a 5% body weight loss (8 LCD, 18 LFD & 12 LGL) of which some were weight gainers. Weight and BMI in this cohort failed to correlate to any measures of insulin resistance but waist

measurements correlated to insulin levels (r= 0.60, 0.52, 0.58, & 0.49; all $p<0.01$) and insulin resistance (r= 0.61, 0.51, 0.59 & 0.52; all $p<0.01$).

Do any of these correlations mean anything? The patterns are variable making it difficult to obtain a clear pattern. Clearly insulin resistance is related to fat distribution and appears to be more responsive to weight loss achieved through diets which have a higher protein, and lower carbohydrate content.

10.9. Discussion of changes in Adipocytokines

Certainly the changes seen within the adipocytokines do not reflect the results of previous studies, but then the number of dietary interventions reporting on these markers are limited and a lot of the theories are extrapolated from animal and laboratory studies rather than clinical scenarios.

Adiponectin and leptin are probably the two most studied as they have been identified for a number of years now. Numerous studies have identified that adiponectin is an anti-inflammatory agent which appears to positively influence. CV risk. It is inversely linked to BMI, waist-hip ratio, insulin resistance, hypertension, dyslipidaemia, and coronary artery disease[87;166;347;398;432].

Pischon in the Health Professionals Follow-Up Study, identified that adiponectin levels were positively affected by alcohol intake and inversely by glycaemic load [424]. The lowest adiponectin levels within our study were certainly within the groups prescribed a low glycaemic load or had restricted carbohydrate intake with instructions to introduce low glycaemic load foods upon completion of the induction phase. These differences were present at baseline and cannot as such be accounted for purely by dietary allocation. Adiponectin did rise as all three groups lost weight with associated improvements in parameters such as insulin resistance and dyslipidaemia. Many studies have reported a correlation between insulin levels, insulin resistance and changes in lipid profile to adiponectin levels. In this study the effect on insulin resistance was somewhat different. Although as a study cohort changes in adiponectin correlated to insulin levels, insulin resistance and negatively with insulin sensitivity, these were only clearly demonstrated among the LCD group. Graphically there was a trend demonstrating the increase in adiponectin levels coinciding with a reduction in insulin levels among the three study groups (Figure 40).

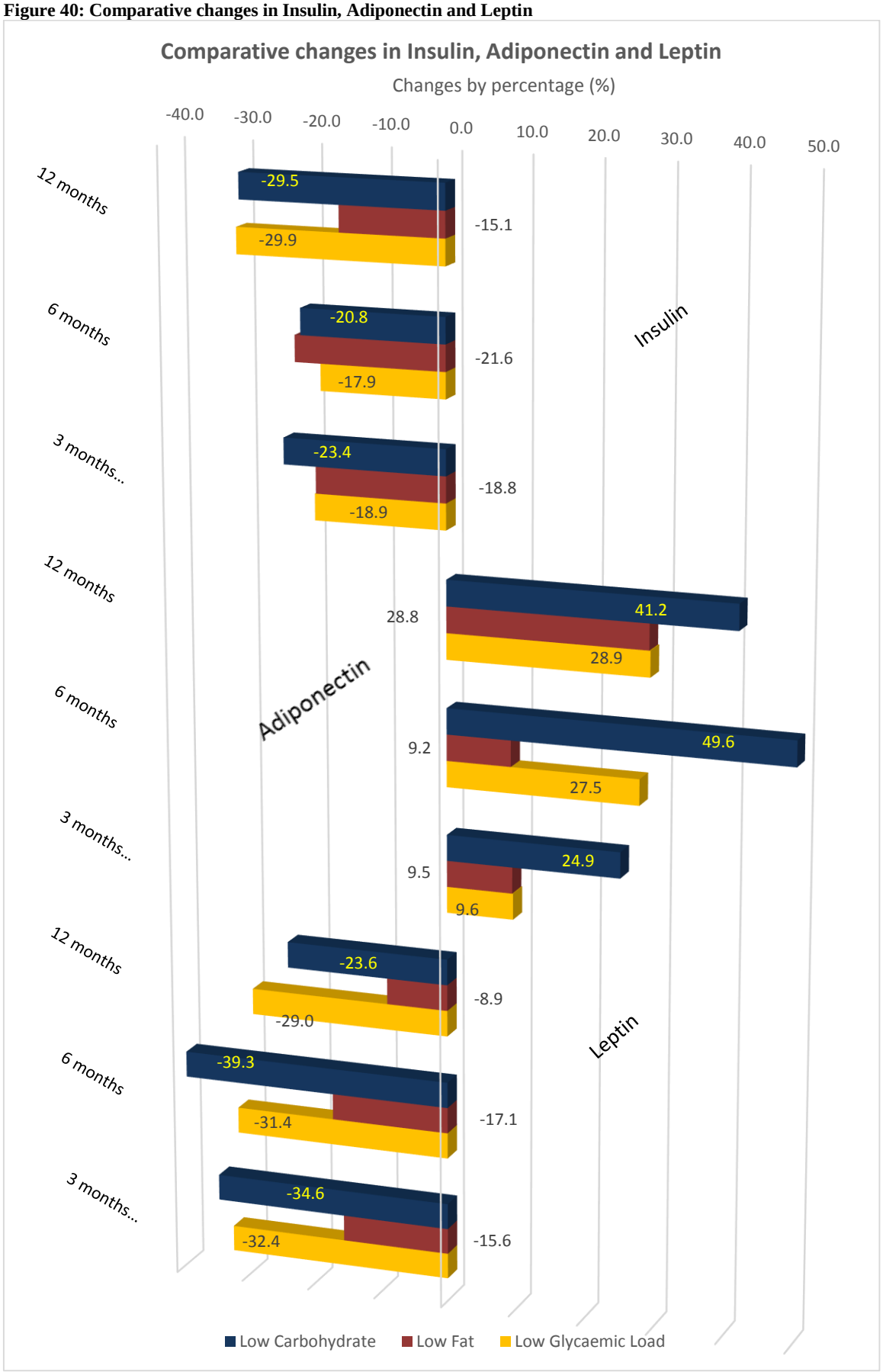
The relationship of adiponectin to both HDL-cholesterol and triglycerides was clearly demonstrated among the three study groups suggesting that these changes are likely to be a general weight loss or caloric restriction effect rather than specific to a particular diet. The HDL-cholesterol and adiponectin relation had previously been

documented in weight loss with lifestyle intervention, lipid studies, risk assessments and chilldren[58;193;279;294;552]. The relation to triglycerides had been observed in laboratory studies and human studies concluding that adiponectin possibly regulated triglyceride and VLDL lipoprotein metabolism[91;350;433]. Therefore, was the reduction seen in triglycerides due to the weight loss or mediated by adiponectin? Certainly the correlations associated the reductions in triglyceride to have been linked to adiponectin specifically and not, weight, BMI or insulin resistance, providing some evidence that adiponectin was a key regulatory factor in VLDL metabolism.

The association between adiponectin and insulin resistance is well established which raises a question to the lack of association noted in the LGL group[218;271;585]. A study in Asian teenagers failed to show a correlation between adiponectin and other cardiometabolic factors and a similar pattern was seen among a group of South Asian females studied in the USA[349;502]. The authors had concluded that variations in ethnicity were responsible for the differences. This cannot explain our findings as the entire cohort was Caucasian.

Other studies have reported that moderate weight loss can improve insulin resistance without affecting adiponectin, but then why was this affect not also noted in the LCD group who lost an equivalent amount of weight[8]. In a review of the effect of dietary intervention on adiponectin, no particular correlation was noted for any dietary macro-nutrient with adiponectin, the only associations appeared to be for weight loss and exercise[497].

Figure 40: Comparative changes in Insulin, Adiponectin and Leptin



As with adiponectin, leptin is well established to be closely linked to obesity, insulin resistance, central adiposity and is used as a predictor of worsening metabolic syndrome[167;496].

The strong relationship demonstrated in this study between leptin and BMI confirms its association with adiposity. It was interesting to note that although both weight and waist measurements correlated to leptin in the study cohort, as individual groups only weight in the LCD group replicated this and the measurement of abdominal circumference, which would have been a better indicator of visceral fat, failed to establish this link. Only BMI demonstrated any correlations when looking at the cohort of those who lost more than 10% body weight and those who failed to reach a 5% weight loss target.

Most studies[49;287;363;564] have demonstrated a strong correlation between leptin, insulin and insulin resistance which appeared to be present in the whole study cohort collectively but failed to be demonstrated in the groups with exception of a small association for fasting insulin levels and a tenuous link to insulin resistance in the LGL arm. The study numbers were small and therefore patterns that became evident when the whole group was analysed as a cohort might have failed to be demonstrated due to the numbers within each study arm.

In addition the study differed from the published evidence in not demonstrating a strong negative correlation to adiponectin. Figure 40 demonstrates that across the three study groups, adiponectin levels tended to reciprocate the changes noted in insulin and leptin.

Human data for resistin are variable with some reporting raised circulating resistin levels in morbidly obese subjects with a positive correlation for BMI and central obesity and others showing no correlation to measures of adiposity[109;118;230;472;496]. The numbers of subjects tested are usually small. Most studies have demonstrated that resistin levels fell with weight loss and that these changes were related to the degree of weight loss with a target of 5% needing to be reached initially[553]. Others believe it is central adiposity which has a greater effect. In a study of 41 females treated with either sibutramine or orlistat and followed for 6 months, the sibutramine group achieved greater weight loss (5.4%) and positively

influenced metabolic parameters as well as displaying significant changes in adipocytokines including resistin. The orlistat group managed a weight loss of 2.5% and failed to show any statistically meaningful changes apart from a superior reduction in resistin levels which was assumed to be due to a comparable reduction in waist circumference[548;553]. Another study of 50 overweight/obese individuals and 20 normal controls (BMI<25kg/m^2) of 4 months duration described an increase in resistin levels in association with weight loss and failed to show an association with measures of insulin resistance or lipid profile[286], while others have failed to show any changes or associations to weight, leading to suggestions that resistin in humans was not as important to visceral adiposity as in rat models[316;380;578]. A cross-sectional study of approximately 240 individuals failed to show that resistin correlated to markers of adiposity, insulin resistance or lipid profile. Resistin levels were not significantly different in the lean, obese, healthy, insulin resistant or in those with type 2 diabetes[316].

In our study resistin behaved differently within each group although the results are compatible with a previous study which had reported no changes in resistin levels following a low carbohydrate diet in 71 obese individuals[252]. Similar results were noted in a study comparing the Lighter-life diet to a low-carbohydrate, high-protein diet. Whereas the Lighter-life group lost a significantly larger degree of weight, resistin levels were no different throughout the nine month study period[456].

Some studies have tried to find a link to variations in resistin levels and macronutrient intake but have failed to show such an association for resistin or adiponectin. One such cross-sectional study studied fasting blood samples, anthropometric measures and 3 day food diaries in 123 healthy Greek students. It reported correlations between circulating leptin and leptin receptors, and anthropometric measures as well as quality of energy intake, but failed to show similar associations with adiponectin or resistin and tentatively concluded by suggesting that a carbohydrate loaded, high glycaemic index diet could lower circulating leptin[592]. The Oslo Diet and Exercise Study studied 187 men randomised to diet only, exercise only, diet and exercise, or control and followed them for a year. It failed to demonstrate any variations in resistin levels in relation to dietary intake or intervention[253].

In addition to its association with insulin resistance, resistin is believed to be associated with inflammation with levels correlating to those of CRP, TNF-α, and interleukin-6[442], a fact that was not replicated within this study despite CRP and PAI-1 levels falling within the LCD and LGL groups, the other measured cytokines which are particularly associated with low grade inflammation.

In this study resistin was noted to positively correlate with visfatin in the LCD and LGL groups. Similar correlations had been noted in other studies involving individuals with and without type 2 diabetes[322;446]. As both these cytokines can be secreted by macrophages, the authors felt that the relationship between the two strengthened the argument for both these cytokines being associated with inflammation, particularly those of a low-grade level such as associated with metabolic syndrome.

Few studies looking at dietary interventions and measurements of visfatin have been published. Our results are similar to recently published studies which have failed to show a correlation between visfatin, dietary intervention or anthropometric measures[10], but they do differ in that visfatin levels rose in all three arms of our study group when it would have been expected to fall in keeping with reductions in visceral adiposity, inflammation and insulin resistance. The changes observed with visfatin in weight loss studies have not been consistent. A group from Spain had studied the effect a three month hypocaloric diet had on visfatin and insulin resistance in a group of 80 obese individuals and reported that visfatin levels fell, but did not show any correlation to insulin resistance[113]. They revisited weight loss in 41 morbidly obese individuals who were placed on a two month hypocaloric diet. They reported that visfatin levels did not change despite a 4.4% average weight loss in this cohort[111].The same group also studied a set of 27 morbidly obese females who had undergone bariatric surgery. The group on average lost 27kg in a year. Despite these changes circulating visfatin levels appeared unchanged[114]. Other studies involving patients who had received bariatric surgery had reported increases in visfatin levels[66;182]. These finding were felt to be contradictory as visfatin is released from visceral fat, which should have decreased in mass with the extreme weight loss secondary to the procedure. The authors were unable to explain why visfatin behaved differently with each group and speculated on whether the changes might be related to the surgical

procedure, particularly as a different group who had undergone gastric banding had demonstrated a reduction in visfatin levels in relation to their weight loss[210], but then visfatin levels have been reported to decrease in a group who underwent bilio-pancreatic diversion. Admittedly this was a group of only 10 females and they had been followed up to 3 years and had lost approximately 50% body weight[345]. Interim data was unavailable to assess whether the changes were due to longevity of time, an effect of the surgery, or the weight loss.

Studies have remarked on visfatin being a positive marker for visceral adiposity. In such circumstances it might be expected to find a relationship between visfatin and waist circumference measurement. As individuals lose abdominal (visceral) fat, it is their waist measurements that decline and certainly these results were successfully achieved with this study. However, contrary to previous publications, visfatin levels actually rose within the first 6 months, the time when weight loss was greatest[29]. A cross-sectional study of 500 subjects failed to demonstrate a correlation between visfatin and height, weight, waist and hip circumferences, waist-to-hip ratio, or blood pressure in both males and females. The only positive associations were with HDL-cholesterol, and a negative correlation to LDL-cholesterol in females[92;599].

Speculation on what causes the variation in the behaviour of visfatin have arisen with some suggesting that as subcutaneous and visceral fat contain differing quantities of this adipocytokine, then potentially the effect may depend on where fat reduction was most affected[407]. Others have suggested that visfatin levels are relatively lower in the morbidly obese as compared to leaner individuals, and in subjects with diabetes as a consequence of the glucose dysregulation. With weight loss and decreasing insulin resistance, visfatin levels rise to return to a normal baseline status[322]. These arguments were strengthened by the fact that rosiglitazone, a known insulin sensitizer increased visfatin levels although the same results were not seen with other hypoglycaemic agents[136;208;243;418].

Weight loss studies looking at the changes in RBP-4 have reported that levels fall with loss of weight in both subjects who have been on diets or those subjected to bariatric surgery[47;80;209].The reductions in RBP-4 are understood to be correlated to changes in BMI and are associated with the degree of weight loss as a minimum

reduction of 5% total body weight was required to reveal any significant changes in circulating RBP-4 levels although there is a belief that adipose RBP-4 would be more likely to show change with these smaller increments of weight loss [256]. Other factors affecting RBP-4 levels were components of metabolic syndrome. A study investigating the changes in RBP-4 in 36 individuals who had been treated with bariatric surgery and then followed up for 2 years confirmed similar reductions as to other weight loss studies with bariatric interventions (16 – 25% reductions in RBP-4)[209;542]. In addition to the changes with weight, they noted that the reductions in RBP-4 levels were greater among those who had the least features of metabolic syndrome.

In this study, although the weight loss achieved was greater than 5%, the changes in RBP-4 levels with exception to the initial 14% reduction in the LCD group were small and not meaningful. At the end of the study RBP-4 levels were no different to the Baseline if not marginally higher.

Correlations with other parameters to RBP-4 were not present with only a tenuous link with the LFD group at the end of the 12 month study period (r= -0.37, $p<0.05$) which is very likely to be an incidental finding as changes throughout the intervals had shown no association. Do the dietary interventions have regulatory roles which affect incretin secretions that indirectly control RBP-4, does bariatric surgery cause alternative modulatory pathways, or is this purely a degree of weight loss effect[195;209;210]? A German group were unable to detect any variation in RBP-4 levels associated to weight or glucose levels and concluded that RBP-4 regulation within humans is potentially different to what has previously been noted in animal studies. The same group had previously reported similarly disappointing results for resistin, but in those circumstances it was not under dietary intervention and they had been looking at gene expression rather than circulating levels assuming these will be interlinked [256].

The data available for PAI-1 are variable with some studies showing a reduction in levels in relation to weight loss and insulin resistance[28], and others failing to show a response[216;348]. The Look AHEAD trial demonstrated a 29% reduction in PAI-1 among their interventional group in comparison to the control where reductions were only 2.5%. These reductions were independent of weight loss and

appeared to be associated with improved glycaemic control, fitness and HDL-cholesterol levels. The authors concluded that these differences potentially indicated improvements in cardiovascular fitness and therefore risk reduction, although this trial was terminated early for failure to show improvements in cardiovascular morbidity and mortality outcomes[57]

Some dietary studies have aimed to see if manipulating various dietary factors will reduce inflammation and therefore the possibility of atherosclerosis and cardiovascular risk. One trial failed to show any change in PAI-1 levels or superiority when comparing a low fat, high fibre diet to conventional therapy in a group of 32 individuals[348]. Another study of 224 healthy middle-aged men studied the effect of triglyceride reduction through supplementation with eicosapentaenoic acid and docosahexaenoic acid as triglycerides levels had been shown to be positively associated with PAI-1 levels. Once more no changes in PAI-1 levels were recorded[216]. In the LIPGENE dietary intervention study, a multi-centre European trial, the investigators compared the effect of manipulating dietary fat on markers of metabolic syndrome and inflammation. Four hundred and eighty individuals were randomised to a high saturated fat diet, a high monounsaturated fat diet, a low fat diet with sunflower oil supplement or a low fat diet with polyunsaturated fat supplements and monitored for twelve weeks. At the end of the study period the use of monounsaturated fats improved triglyceride levels but had no effect on PAI-1 levels[532].The use of a low glycaemic load as compared to conventional low fat therapy was found to improve PAI-1 levels in a study by Ebbeling[132].

Therefore positive changes seen in PAI-1 particularly in the first half of the study is difficult to relate directly to diet and is likely to be associated with the other favourable changes which were observed including improvements in insulin sensitivity, reductions in triglyceride levels and possibly the weight loss to an extent. Despite weight loss being maintained within the three groups, PAI-1 levels in the LFD arm rose to almost baseline at the end of the study. The results from this study have shown that the changes in both insulin and triglyceride levels were more prominent and sustained in the LCD and LGL groups, and this could be a major reason for the maintained improvement of PAI-1 levels within the two diet groups.

Could this be an effect of the higher carbohydrate load in LFD or as noted by Ebbeling, does a low fat diet have no effect on this marker of inflammation?

HsCRP levels are well documented to be elevated in inflammatory conditions as well as obesity and insulin resistance. A trial in 38 obese females looking at the relationship between weight, insulin resistance and hsCRP concluded that hsCRP levels fell in association with improvements in insulin resistance and not weight reduction[361]. This may explain the improvements in hsCRP seen in the study for the LGL and LCD groups, but what explains the large rise noted in the LFD group at twelve months? Being a marker of acute stress and inflammation outside factors could potentially have affected the outcomes. Other weight loss studies have related the reduction in hsCRP directly to the weight loss[530].

A study by an Italian group looked at 180 subjects who were randomized to either a Mediterranean style diet or a low fat diet and were followed for two years. At the end of the study the Mediterranean arm had lost more weight, abdominal fat, showed superior improvements in lipid profile, and reductions in hsCRP and other markers of endothelial dysfunction. In addition to these improvements, there was demonstrated improvements in the criteria that make up the metabolic syndrome. It was the effect of these improvements, which allowed the study group to conclude that markers of MS were factorial in variations in hsCRP[138].

A recent systematic review of dietary interventions and the effect they had on adipocytokines studied approximately 50 studies which involved dietary interventions only, excluding those which included exercise, pharmacological agents, or surgical interventions. Weight loss varied between 0.8–20percent of total body weight. They reported that leptin levels decreased uniformly in all but three of the studies and was a good marker of residual fat mass. Adiponectin levels did not appear to be affected by dietary intervention with only seven reporting a rise and one showing a decrease. RBP-4 and visfatin fell in the studies where they had been measured and PAI-1 as well as other inflammatory adipokines showed mixed results. The authors concluded that apart from leptin the results from other adipocytokines were too variable to allow characteristic behaviour of the cytokines to be defined particularly in response to diet. Of note the classifications used by the authors for the diets were low calorie, very low calorie or weight maintenance programmes rather

than examining the dietary macronutrient content[284]. This review along with an interventional weight loss study using pharmaceutical agents (orlistat or sibutramine) on cardiometabolic markers and adipocytokines.[548] have supported the findings in our own study, which demonstrated reductions in weight, waist circumference, and systolic blood pressure. There was a rise in HDL-cholesterol and fall in triglycerides (Table 50). Improvements in insulin resistance were demonstrated (Table 51). With regards to adipocytokines we demonstrated a rise in adiponectin, with a corresponding fall in leptin. Visfatin and RBP-4 levels were static or rose, and PAI-1 levels fell (Table 52).

Some of the change in the various adipocytokines, were not predictable, and did not conform to previous findings. In addition, despite what appeared to be significant improvements in metabolic parameters, no apparent correlations between the cytokines existed.

Table 50: Changes in anthropometric measures and lipid profiles among the whole study cohort

	Mean	Std. Deviation
Weight (kg)		
Baseline	97.6	17.3
3months	91.6♥	16.1
6months	90.4♥	16.7
12months	91.1♥	16.8
BMI (kg/m^2)		
Baseline	34.4	5.4
3months	32.3♥	5.1
6months	31.9♥	5.3
12months	32.2♥	5.5
Waist circumference (cm)		
Baseline	108.1	11.1
3months	101.5♥	10.7
6months	99.0♥	11.1
12months	98.0♥	11.6
Systolic BP (mmHg)		
Baseline	139.7	15.3
3months	136.4♦	16.3
6months	133.5*	16.5
12months	133.2♥	16.1
Diastolic BP (mmHg)		
Baseline	84.7	9.1
3months	84.0	8.5
6months	80.9♦	8.0
12months	80.7♥	7.9
Total cholesterol (mmol/l)		
Baseline	5.7	0.9
3months	5.6♦	1.0
6months	5.7	1.1
12months	5.7	1.0
HDL-cholesterol (mmol/l)		
Baseline	1.4	0.4
3months	1.4	0.4
6months	1.5*	0.5
12months	1.6♥	0.5
LDL-cholesterol (mmol/l)		
Baseline	3.5	0.8
3months	3.5	0.9
6months	3.5	1.0
12months	3.4	0.9
Triglycerides (mmol/l)		
Baseline	2.0	0.9
3months	1.5♥	0.7
6months	1.5♥	0.7
12months	1.6♥	0.8

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to changes from baseline)

Table 51: Changes in insulin and insulin resistance among the study cohort

	Mean	Std. Deviation
Fasting glucose (mmol/l)		
Baseline	5.8	1.6
3months	5.4♦	1.0
6months	5.3*	1.1
12months	5.5♦	1.3
Insulin (mIU/ml)		
Baseline	19.0	10.5
3months	14.6*	8.0
6months	14.1♥	8.0
12months	13.5♥	6.9
HOMA2 IR		
Baseline	2.5	1.4
3months	1.9*	1.0
6months	1.8♥	1.0
12months	1.7♥	0.9

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to changes from baseline)

Table 52: Changes in Adipocytokines among the study cohort

	Mean	Std. Deviation
Adiponectin (ng/ml)		
Baseline	8592	6504
3months	9367♦	7432
6months	10604*	8538
12months	10736♥	7711
Leptin (ng/ml)		
Baseline	31.5	22.2
3months	22.4♥	17.7
6months	21.9♥	17.1
12months	24.8♥	20.6
Resistin (ng/ml)		
Baseline	10.6	3.4
3months	10.6	2.6
6months	10.8	3.0
12months	9.7♦	2.7
Visfatin (ng/ml)		
Baseline	1468	1186
3months	2133♦	1760
6months	2495	2066
12months	1887	1696
RBP4 (mcg/ml)		
Baseline	87.3	35.3
3months	88.2	31.8
6months	90.8	42.3
12months	96.0	44.1
PAI-1(ng/ml)		
Baseline	78.6	53.3
3months	61.8	50.5
6months	50.7*	29.0
12months	58.2*	79.1
CRP (ng/ml)		
Baseline	5688	8936
3months	4715	5443
6months	4494	5716
12months	4548	8481

♦ p< 0.05, * p <0.01, ♥ p <0.001 (p-values refer to changes from baseline)

11. CHAPTER 11: Concluding Remarks

In the trial described in this thesis the study cohort resulted in a clinically significant reduction in weight with improvements in fasting glucose, insulin levels, insulin resistance, and triglycerides (Table 50-Table 52).

All three dietary approaches have produced clinically meaningful improvements in the primary outcome, weight as well as in anthropometric measures.

Changes in the secondary outcomes demonstrated a reduction in the prevalence of metabolic syndrome across the three groups with the greatest change noted within the LCD group in all 5 components of MS. LFD changes were conservative with the number of individuals with impaired fasting glycaemia unchanged and only a modest fall in those with systolic blood pressures >130mmHg.

Positive outcomes in the lipid profile was most prevalent within the LGL group where there were improvements in HDL-cholesterol, LDL-cholesterol and triglycerides. Positive changes in triglycerides and HDL-cholesterol were demonstrated among the LCD group but not LDL-cholesterol.

The improvements in fasting glucose, insulin levels and markers of insulin resistance among the three groups were clinically meaningful. The changes in glucose and insulin were particularly maintained within the LGL and LCD groups, implying that the reduced refined carbohydrate load recommended by both these regimens may exert additional benefits in improving insulin resistance compared to LFD diets.

These benefits are the reduction in insulin resistance, and calculated improvement in ß-cell function which in the long term may help delay, if not preserve ß-cell function, and potentially, prevent the progress to diabetes in these MS individuals.

The changes noted among the tertiary outcomes, adipocytokines, were variable with some following patterns demonstrated in previous studies and others failing to provide any form of concordance.

The study period was only 12 months and although adequate to help reflect some short and medium term outcomes cannot reflect long term changes, particularly cardiovascular outcomes which are important. The subject numbers were relatively small. Longer term follow-up is required to see whether the reductions in circulating insulin and insulin resistance may be maintained along with weight loss maintenance, and to assess the impact this would have on other cardiovascular risk factors.

A reduction in leptin and an increase in adiponectin in response to weight changes and improvements in insulin resistance were seen in all the groups. Correlations (between cytokines and weight change) were not always clearly demonstrated although trends followed patterns seen in other dietary studies.

Low-fat diets as currently advised for healthy lifestyles certainly do result in a modest degree of weight loss with some improvement in parameters making up the metabolic syndrome, but changes are modest and potentially lead to early dissatisfaction and poor compliance. In this study a low fat diet had a small positive effect on serum triglycerides and HDL-cholesterol, changes which are likely to be weight related as they were seen across the groups but in greater effect with the other regimens.

Of concern was the effect seen on blood pressure within the LFD group. The reduction in systolic blood pressure were the lowest in the LFD group despite its participants requiring more up-titration of anti-hypertensive therapy than the other two groups. They were also the group with the greatest reduction in salt intake as well as the least overall salt intake within the study period. Although diastolic blood pressure reductions were greatest with the LFD the importance of this alone in cardiovascular risk protection is unclear. Certainly LFD failed to demonstrate any benefits in CVD and CHD risk reduction through standard risk calculations.

Compliance on the LFD was generally good and weight appeared to be maintained throughout the study period making it suitable for those who may seek a weight maintenance method. Regardless of current recommendations we have

certainly failed to demonstrate an advantage over other methods for this particular diet for those individuals who are seeking to reduce their cardiovascular risk.

A high-fat, low-carbohydrate approach did result in the greatest reductions in weight among the groups, with most of individuals who were considered the best weight losers being among this group. This diet positively influenced such markers as HDL-cholesterol, triglycerides, systolic blood pressure, and improvements in insulin resistance which was reflected in the reductions in calculated 10 year risks for cardiovascular disease. In addition, if the high fat intake could be modified to types which are potentially helpful (i.e. the monounsaturated fats benefits may be more attractive).

The strict carbohydrate measures within the LCD diet meant it encouraged early failure with withdrawal, among those randomised to it, due to the inability to follow the carbohydrate restrictions. In individuals motivated to succeed, weight reductions were significant although long term maintenance may be a challenge.

The LCD group showed favourable effects on insulin levels and insulin sensitivity. It demonstrated steady improvements in inflammatory markers and cytokines with the exception of resistin and RBP-4. The lack of overall change in those two cytokines was a trend that was seen in all the dietary groups.

LCD diets, although controversial should not be completely discounted, as numerous studies including this one, have identified many parameters which these diets have positively affected. As a diet to facilitate the start of weight loss, there appears to be no reason why they should not be used as long as contraindications such as renal dysfunction have been assessed. Evidence has shown that their benefits are mainly within the first six months and this may be where they should be considered. Concordance is poorer than for other dietary regimens and weight is generally regained quickly unless individuals are able to maintain some form of controlled carbohydrate intake.

A diet low in glycaemic load certainly appears to incorporate the best qualities for most parameters. It appeared to be tolerable with drop-outs being equal to those on a low fat diet. Concordance to diet was reasonable with the only issues being

meeting the criteria for intake of monounsaturated fats. In the study these individuals were encouraged to use spreads that were higher in monounsaturated fats but the polyunsaturated or saturated fats were still the major components in those products. The use of natural products (i.e. olive oil or rapeseed oil) rather than "Clover" or "Bertoli" is likely to help improve the quality of fat intake and positively influence this dietary regimen

The LGL group was effective at weight reduction despite the highest overall average calorie intake of the groups, improvements in insulin sensitivity, blood pressure reduction, as well as overall improvements in the lipid profile. Those on LGL were the only group to demonstrate reductions in total and LDL-cholesterol with improvements in the HDL-cholesterol to total cholesterol ratio that was equal to that seen in the LCD group. These positive changes were reflected in the reductions in the 10 year cardiovascular and coronary heart disease risk calculations.

The effect of LGL on cytokines and sub-clinical inflammation is unclear. The trends demonstrate improvements in these markers although many of the changes were not significant.

As part of a lifestyle intervention programme for cardiovascular risk modification for the overweight or insulin resistance, a low glycaemic load or Mediterranean approach is certainly a favorable regimen for nutritional intake and even weight loss. It provides improvements in all markers of cardiovascular disease, and favourable changes in inflammatory markers. The nutritional intake is varied avoiding the strict restrictions in the LCD group which was a limiting factor for compliance and long term maintenance.

The Look AHEAD trial was discontinued for failing to demonstrate the benefits of an intense lifestyle intervention on cardiovascular morbidity and mortality. The dietary interventions used were the recommended standards with the intensification being through regular follow up and other life-style changes including exercise. A long term cardiovascular outcome trial studying the effect that different dietary programmes exert on these parameters would be of importance for the development of dietary recommendations that are more strongly evidence based.

12. Appendix:

Figure 42: GP Invitation Letter

Wolfson Centre,
Royal United Hospital,
Combe Park,
Bath, BA1 3NG

Dr A N Other,
20 High Street,
Another Town
Post Code

Date

Dear Dr Other

A Randomised Study comparing the effect of 3 dietary approaches on Cardiovascular Risk in Subjects with the Metabolic Syndrome

The prevalence of the metabolic syndrome within the UK is steadily increasing with over 16% of adult men and women fulfilling the diagnosis. Associated are various components that collectively or individually increase the risk of cardiovascular disease (Type 2 Diabetes, hypertension, obesity and dyslipidaemia).Studies have proposed that early and aggressive treatment of the metabolic syndrome decreases these risks significantly and we are becoming increasingly obliged to intervene early. Management can take the form of various methods i.e. diet, exercise, and oral therapy.

We are particularly interested at investigating the diet aspect of management and are currently undertaking a randomised controlled study comparing the effect of three differing diets on cardiovascular risk in subjects with the metabolic syndrome.

The study will be over a period of 6 months with a potential extension to a year. Subjects will be seen at the Wolfson Centre, Royal United Hospital.

During this time individuals will be counselled to follow one of three dietary approaches and seen at regular intervals to assess their compliance, make adjustments to their dietary regimes or for a physical examination and blood tests including fasting glucose, lipids, urea and electrolytes, and liver functions.

We are looking to recruit men and women aged between 18 – 75 years who will fit the diagnosis of the metabolic syndrome – i.e. they should fulfil 3 of the 5 following criteria-

1. abdominal obesity (waist circumference >102cm (40in) in men and >88cm 35in) in women)
2. triglyceride level >150mg/dL (1.7mmol/L)
3. HDL cholesterol <40mg/dL (1mmol/L) for men and <50mg/dL (1.3mmol/L) for women
4. Blood pressure >130mm systolic or 85mm diastolic
5. fasting plasma glucose > 110mg/dL (6.6mmol/dL)

Please may you contact Dr Rasha Mukhtar at the Wolfson Centre on 01225 824127/ 824125 with the details of any suitable subjects. Alternatively you may e-mail the details to mpxrm@bath.ac.uk.

Thank you for your co-operation and I look forward to hearing from you soon.

Dr J P D Reckless, Dr A M Robinson, Dr E R Higgs,
Dr R Y A Mukhtar
Diabetes and Lipid Research, Wolfson Centre, Royal United Hospital

Figure 43: Patient Invitation Letter

Mr/ Ms A N other
House number
Street
City
Post code

Date

Dear Mr/Ms Other

I am writing to invite you to take part in a research study looking at the effect of different diets on weight and on the factors that might increase the risk of developing a heart attack. We are looking for people who may fulfil the criteria of the metabolic syndrome (assessed by blood pressure, waist measurements, cholesterol and fatty acid levels, and blood sugar readings) and have some of the risk factors that can go with an increased risk of heart disease, stroke or associated complications.

This study is researching three different weight-reducing diets, to see as weight loss occurs if they have different effects on blood pressure and on blood sugar and cholesterol levels

Taking part in the study is entirely up to you and your future care will not be affected by your choice. If you are interested in taking part, then we would ask you to come up to the Wolfson Centre at the Royal United Hospital to discuss the study and answer any queries. We will be able to provide you with transport and reasonable travel expenses for your visits if required.

I have taken the liberty to include a copy of the study information sheet (criteria on it do not necessarily describe you), consent form and a questionnaire that will assist us in seeing if you are eligible.

Should you agree to the study then please return the signed consent form and completed questionnaire in the enclosed self-addressed envelope so that we may contact you with an appointment or to discuss the study further.

If not interested then please indicate so on the questionnaire without completing the medical part so that we do not disturb you again.

I look forward to hearing from you.

Dr Rasha Mukhtar
Research Registrar, Wolfson Centre, Royal United Hospital on behalf of
Dr W H Other at Somewhere Else Surgery and
Dr John Reckless, Dr Tony Robinson and Dr Lynn Higgs at the Royal United Hospital

Figure 44: Initial details form

A Randomised Study comparing the effect of three dietary approaches on Cardiovascular Risk in Subjects with the Metabolic Syndrome

Name:–_____

Date of Birth _____**Sex**_____

Contact Details_____

_____

_____

_____

Please delete as appropriate -

I am interested in taking part in this study /

I am not interested in taking part in this study.

Do not complete following unless you intend to take part in this study.

Height	**Weight**	
Do you have	**Diabetes**	**Yes / No / Do not know**
	Hypertension	**Yes / No / Do not know**
	Angina	**Yes / No / Do not know**
	High Cholesterol	**Yes / No / Do not know**

Please list your current medication-

Are you a vegetarian? **Yes / No**

Do you have any allergies? **Yes / No**

If yes then please list what allergies

Figure 45: Consent Form

Patient Consent

A Randomised Study comparing the effect of three Dietary Approaches on Cardiovascular Risk in Subjects with the Metabolic Syndrome

Please initial each of the boxes

1. I confirm I have read and understand the patient information sheet. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected ☐

3. I am willing to allow access to my medical records but understand that strict confidentiality will be maintained. ☐

4. I agree to take part in the above study. ☐

5. I agree for my GP to be notified ☐

Name of Patient	**Date**	**Signature**
Name of person taking consent (if different from researcher)	**Date**	**Signature**
Researcher	**Date**	**Signature**

Table 53: Baseline Dietary Questionnaire

Dietary Questionnaire

The aim of this questionnaire is to help us understand your current eating habits. We can then guide you on the dietary changes that you will need to make to participate in the study.

How often do you eat the following foods (put a tick in the appropriate box):

Food	Most days	2-3 times a week	Once a week or less	Once a month or less	Never
Potatoes					
Sweet Potato					
Pasta					
Rice					
Couscous					
Crispbreads such as ryvita					
Noodles					
Oven chips					
Home cooked chips in deep fryer					
Fruit					
Vegetables (excluding potatoes)					
Yogurt					
Hard Cheese					
Cheese spread					
Cottage cheese					
Oily Fish (salmon, pilchards, sardines, mackerel, trout)					
White Fish (ie cod etc)					
Baked beans					

Lentil or pulses					
Nuts					
Beef					
Lamb					
Chicken					
Turkey					
Duck					
Sausages					
Burgers					
Mince					
Quorn					
Tofu					
Commercially prepared cooking sauces					
Ready meals					
Crisps					
Cake					
Biscuits					
Chocolate					
Sweets					
Ice cream					
Cream					

1. **Do you generally eat 3 meals a day?**
    a. Yes No

2. **If No which meal(s) do you often skip (circle one or more)**
    a. breakfast
    b. midday meal
    c. evening meal

3. **How often do you have snacks during the day between meals such as mid-morning, mid-afternoon, or a supper in the evening.**
    a. Most days Occasionally Rarely Never

4. **Do you do the food shopping?**
    a. Yes
    b. No, if not who does:_____

5. **Do you usually do the cooking?**
    a. Yes
    b. No, if not, who does:_____

6. **What type of bread to you mostly use (please circle):**
    a. White bread
    b. Brown bread
    c. Wholemeal bread
    d. Granary bread (with visible grains)
    e. White bread with added fibre
    f. Other, please specify:_____

7. **If you eat breakfast cereals, name the brand(s) you normally eat:_____**

8. **What type of milk do you drink:**
    a. Whole milk (blue top)
    b. Semi skimmed (green top)
    c. Skimmed milk (red top)
    d. Soya milk
    e. Other, please specify:_____

9. **How much milk do you think you would drink on an average day :_____**

10. **If you eat yogurt what type do you usually buy (name the brands):_____**

11. **Do you usually cut the fat off the meat:**
    a. Yes, before cooking
    b. Yes, after cooking
    c. No

12. **How many eggs would you normally eat each week:_____**

13. **What type of margarine or fat spread to you usually use: _____**

14. **What type of oil or fat do you use in cooking:**
    a) Sunflower oil (vegetable oil)
    b) Olive oil
    c) Rapeseed oil (vegetable oil)
    d) Peanut oil
    e) Lard
    f) Butter

15. **Do you drink alcohol**

    Yes No

16. **If YES, please indicate how much of each of these alcoholic beverages you would have on a typical week.**

    a. Beer/Lager/Cider : _____ pints
    b. Red Wine _____ glasses
    c. White Wine _____ glasses
    d. Spirits _____ nips
    e. Sweet Liquors _____ nips

17. **How often do you buy your lunch out?**
    a. Most days
    b. 2-3 times a week
    c. once a week or less
    d. once a month or less

18. **How often to you buy takeaway foods (such has pizza, fish and chips, Chinese, Indian, McDonalds etc)**
    a. Most days
    b. 2-3 times a week
    c. once a week or less
    d. once a month or less

19. **How often do you eat out at a restaurant, pub, café etc)**
    a. Most days
    b. 2-3 times a week
    c. once a week or less
    d. once a month or less

20. **How often do you eat at other peoples homes (ie family, friends etc):**
    a. Most days
    b. 2-3 times a week
    c. once a week or less
    d. once a month or less

Thank you for taking the time to complete this. Please bring this with you to your next visit to the Wolfson Centre.

Table 54: Schedule of events from screening to end

WEEK	NEXT VISIT	PHYSICAL EXAM	COMPLIANCE	WEIGHT	WAIST	BP	OGTT	FASTING BLOODS	WEIGHED FOOD RECORD	OTHER
-2 (screening)		X		X	X	X			7 days (food scales)	• Consent • Medical history • Physical examination • Food questionnaire
0 (randomisation)				**X** (height)	**X**	**X**	**X**	**X**		• Randomised to dietary plan, info given • Food diary
2			**X**	**X**	**X**					• Food diary
4			**X**	**X**	**X**					• Food diary
6			**X**	**X**	**X**				4 days (food scales)	•
8			**X**	**X**	**X**					• Food diary
10			**X**	**X**	**X**					• Food diary
12		**X**	**X**	**X**	**X**	**X**		**X**	3days (food scales)	•
14			**X**	**X**	**X**					• Food diary
16			**X**	**X**	**X**					• Food diary
18			**X**	**X**	**X**					• Food diary
20			**X**	**X**	**X**					• Food diary
22			**X**	**X**	**X**					• Food diary
24		**X**	**X**	**X**	**X**	**X**	**X**	**X**	3 days (food scales)	•
28			**X**	**X**	**X**					• Food diary
32			**X**	**X**	**X**					• Food diary
36			**X**	**X**	**X**					• Food diary
40			**X**	**X**	**X**					• Food diary
44			**X**	**X**	**X**					• Food diary
48			**X**	**X**	**X**					• Food diary
52		**X**	**X**	**X**	**X**	**X**	**X**	**X**	3 days (food scales)	•

Table 55: Table of Prescribed Energy Intake

Male:-

PRESCRIBED ENERGY INTAKE

18-30 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-65	9' 6 - 10' 5	1500	1800	2200	2200
66-70	10' 6 - 11' 1	1500	2000	2200	2200
71-75	11' 2 - 11' 12	1500	2000	2200	2200
76-80	11' 13 -12' 9	1800	2200	2200	2200
81-85	12' 10 - 13' 6	1800	2200	2200	2200
86-95	13' 7- 15' 0	2000	2200	2200	2200
96-110	15' 1- 17' 5	2200	2200	2200	2200
111-125	17' 6 - 19' 10	2200	2200	2200	2200
126-135	19'11 - 21' 4	2200	2200	2200	2200
>135	> 21' 4	2200	2200	2200	2200

Light Activity: Some daily activity (at work or tasks about the house or garden) with at least 2 hours on their feet.

31-60 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-65	9' 6 - 10' 5	1500	1800	2200	2200
66-70	10' 6 - 11' 1	1500	2000	2200	2200
71-75	11' 2 - 11' 12	1500	2000	2200	2200
76-80	11' 13-12' 9	1800	2000	2200	2200
81-85	12' 10 - 13' 6	1800	2200	2200	2200
86-90	13' 7- 14' 3	1800	2200	2200	2200
91-110	14' 4- 17' 5	2000	2200	2200	2200
111-120	17' 6- 18' 13	2200	2200	2200	2200
121-150	19'0– 23'9	2200	2200	2200	2200
151-160	23'10-25'4	2200	2200	2200	2200
>160	> 25'4	2200	2200	2200	2200

Moderate Activity: Assumes 6 hours on their feet or regular strenous exercise.

Over 60 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-65	9' 6 - 10' 5	1200	1500	1800	2000
66-70	10' 6 - 11' 1	1200	1500	1800	2200
71-75	11' 2 - 11' 12	1200	1500	2000	2200
76-80	11' 13 -12' 9	1500	1800	2000	2200
81-85	12' 10 - 13' 6	1500	1800	2200	2200
86-95	13' 7- 15' 0	1500	2000	2200	2200
96-100	15' 1- 15'11	1800	2000	2200	2200
101-110	15' 12-17'5	1800	2200	2200	2200
111-125	17' 6- 19'10	2000	2200	2200	2200
126-135	19' 11 - 21' 4	2200	2200	2200	2200
136-165	21' 5- 26' 1	2200	2200	2200	2200
>165	> 26' 1	2200	2200	2200	2200

Heavy: Those in heavy labouring jobs or serious athletes in training.

Female

18-30 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-70	9' 6 -11' 1	1200	1500	1500	2000
71-75	11' 2 - 11' 12	1500	1800	2000	2300
76-80	11' 13 - 12 ' 9	1500	2000	2000	2300
81-85	12' 10 - 13' 6	1500	2000	2000	2500
86-90	13' 7- 14, 3	1500	2000	2200	2200
91-100	14' 4 - 15 ' 11	1800	2200	2200	2200
101-114	15 ' 12 - 18 '0	2000	2200	2200	2200
115-125	18'1- 19' 10	2200	2200	2200	2200
>125	>19 ' 10	2200	2200	2200	2200

Light Activity: Some daily activity (at work or tasks about the house or garden) with at least 2 hours on their feet.

31-60 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-70	9' 6-11' 1	1200	1500	1500	1800
71-75	11' 2- 11' 12	1200	1500	1800	2000
76-80	11' 13- 12 ' 9	1200	1800	1800	2000
81-95	12' 10 - 15'0	1500	1800	2000	2200
96-114	15'1 -18'0	1500	2000	2200	2200
115-130	18' 1- 20' 7	1800	2200	2200	2200
131-150	20' 8 - 23' 9	2000	2200	2200	2200
151-170	23' 10- 26' 12	2200	2200	2200	2200
>170	> 26'12	2200	2200	2200	2200

Moderate Activity: Assumes 6 hours on their feet or regular strenous exercise.

Over 60 years:

Wt (kg)	Wt (Stone)	Inact	Light	Mod	Heavy
60-65	9' 6- 10' 5	1200	1200	1500	1500
66-75	10' 6 - 11' 12	1200	1500	1500	1800
76-85	11' 13 - 13' 6	1200	1500	1800	2000
86-95	13' 7- 15'0	1500	1800	2000	2000
96-100	15' 1 -15 ' 11	1500	2000	2000	2200
101-110	15 ' 12 - 17' 5	1500	2000	2000	2200
111-120	17' 6- 18' 13	1500	2000	2200	2200
121-135	19'0 - 21' 4	1800	2200	2200	2200
136-165	21' 5 - 26'1	2000	2200	2200	2200
>165	> 26'1	2200	2200	2200	2200

Heavy: Those in heavy labouring jobs or serious athletes in training.

Figure 46: The four phases of Atkins

A brief summary of the phases of the Atkins diet

1. Phase 1: Induction

This is the period where the body generates energy through the burning of fat and therefore results in ketosis. It is expected to be a minimum period of fourteen days but can be extended for longer. Carbohydrate intake is restricted to no more than 20gms per day (e.g. 120gm of salad vegetables). Meat, fish, shellfish or poultry, fats and eggs are allowed to be taken unrestrictedly. Cheese is limited initially as it does contain milk products. Individuals are encouraged to drink plenty of fluid in the form of water and avoid large quantities of caffeine which may potentially induce low blood glucose. Atkins claims that the induction phase helps regulate blood glucose levels, improve satiety and curb the cravings for sugary substances.

2. Phase 2: Ongoing weight loss

Ongoing weight loss is the stage where ketosis is ongoing but, there is a gradual increase in the daily carbohydrate allowance. Individuals are expected to introduce more variety of salads and low glycaemic load products such as yoghurts, nuts and possibly berry fruits. The recommendations is that the carbohydrate intake is increased by 5gms per week until a point is reached where weight loss has slowed down but continues at a steady and satisfactory rate. This phase is expected to allow the dieter understand what their carbohydrate threshold is, allow them to experiment with a wider variety of food products, adopt alternatives for favourite carbohydrate products and prepare them for long term weight management. Ongoing weight loss can continue for many months until an individual feels they have achieved their target weight.

3. Phase 3: Pre-maintenance

In the third phase, carbohydrate intake continues to gradually rise to the point where weight loss stops, "critical carbohydrate level for maintenance" a point above which any further carbohydrate intake is likely to cause weight gain. It has been designed to allow the gentle transition into ongoing weight maintenance and avoid an individual returning to their previous eating habits. By this point dieters would be expected to

have become accustomed for their carbohydrate intake to be composed of nuts, seeds, berries, low carbohydrate fruit and vegetables, some milk products and legumes.

4. Phase 4: Life time maintenance

The final stage of the diet is life time maintenance by which an individual will have identified their carbohydrate allowance, and have reached their target weight loss. Refined carbohydrates should have been eliminated from the individual's intake and healthier, low glycaemic load carbohydrates introduced instead.

There will be times that weight starts rising and the general advice from the books appears to be returning to ongoing weight loss and rebuilding the steps.

Table 56: List of allowed foods in Atkins adapted from [40]

Examples of foods allowed on Atkins

All fish including:

- Flounder
- Herring
- Salmon
- Sardines
- Sole
- Tuna
- Trout
- Cod
- Halibut

All fowl including:

- Cornish hen
- Chicken
- Duck
- Goose
- Pheasant
- Quail
- Turkey
- Ostrich

All shellfish including:

- Clams
- Crabmeat
- Mussels*
- Oysters*
- Shrimp
- Squid
- Lobster
- *Oysters and mussels are higher in carbs

All meat including:

- Bacon*
- Beef
- Ham*
- Lamb
- Pork
- Veal
- Venison

Some processed meat, bacon, and ham is cured with sugar

Eggs in any style

Cheese	Serving Size	Grams of net carbs
Blue cheeses	2 T	0.4
Cheddar	1 oz	0.4
Cow, sheep and goat	1 oz	0.3
Feta	1 oz	1.2
Gouda	1 oz	0.6
Mozzarella	1 oz	0.6
Parmesan	1 oz	0.9
Swiss	1 oz	1.0

Vegetable	Serving Size/Prep	grams of net carbs
Alfalfa sprouts	½ cup/raw	0.2
Bok Choy	1 cup/raw	0.4
Celery	1 stalk	0.8
Chives	1 tablespoon	0.1
Cucumber	½ cup	1.0
Iceberg lettuce	1 cup	0.2
Mushrooms	½ cup	1.2
Parsley	1 tablespoon	0.1
Peppers	½ cup/raw	2.3
Radicchio	½ cup/raw	0.7
Radishes	6/raw	0.5
Romaine lettuce	1 cup	0.4

Vegetables:

You should be eating approximately 12 to 15 grams of net carbs per day in the form of vegetables, which is equivalent to several cups depending on the actual carb content of the veggies you select.

Vegetable	Serving Size/ Prep	Net Carbs
Artichoke	1/2 medium	3.5
Asparagus	6 spears	2.4
Avocados	½ whole (raw)	1.8
Bamboo shoots	½ cup	1.2
Broccoli	½ cup	1.7
Broccoli raw	½ cup	0.8
Brussels sprouts	¼ cup	1.8
Cabbage	½ cup (raw)	1.6
Cauliflower	½ cup (raw)	1.4
Eggplant	½ cup	2.0
Green String Beans	1 cup	4.1
Hearts of palm	1 heart	0.7
Leeks	½ cup	3.4
Olives green	5	0.1
Olives black	5	0.7
Onion	¼ cup	4.3
Pumpkin	¼ cup	2.4
Rhubarb	½ cup (unsweetened)	1.7
Sauerkraut	½ cup (drained)	1.2
Snow peas and snap peas in pod	½ cup with pods	3.4
Spinach	½ cup	2.2
Tomato	¼ cup	4.3
Turnips	½ cup	3.3
Zucchini	½ cup	1.5
Sage	1 tbs	0.0
Tarragon	1 tbs	0.0

Salad Dressings - Any prepared salad dressing with no added sugar and no more than 2 grams of net carbs per serving (1-2 tablespoons) is acceptable. Or make your own.

Blue cheese	2 tbs	2.3
Caesar	2 tbs	0.5
Italian	2 tbs	3.0
Lemon juice	2 tbs	2.8
Lime juice	2 tbs	2.8
Oil and vinegar	2 tbs	1.0

Fats and Oils

There are no carbs here, but keep in mind that the serving size is approximately 1 tablespoon.

1. Butter
2. Mayonnaise – make sure it has no added sugar
3. Olive oil
4. Vegetable oils – Those labelled "cold pressed" or "expeller pressed" are especially good and olive oil is one of the best.
   - Canola*
   - Walnut
   - Soybean*
   - Grape seed*
   - Sesame
   - Sunflower*
   - Safflower*

Do not allow any oils to reach overly high temperatures when cooking. Use olive oil for sautéing only. Use walnut or sesame oil to dress cooked veggies or salad, but not for cooking.

Artificial Sweeteners

- Splenda – one packet equals 1 gram of net carbs

Beverages

- Clear broth/ bouillon (make sure it has no sugars added)
- Club soda
- Cream, heavy or light.
- Decaffeinated or regular coffee and tea – one two cups only
- Diet soda (be sure to note the carb count)
- Flavoured seltzer (must say no calories)
- Herb tea (without added barley or fruit sugar added)
- Unflavoured soy/almond milk
- Water – at least eight 8-ounce glasses per day

Figure 47: Copy of the eating plan given to those on the LGL Diet

Metabolic Plan

You have been selected to participate in this study as you have a number of risk factors for disease such as high blood pressure, cholesterol, larger than ideal waist measure, and higher sugar levels.

You have been randomly allocated to follow the **Metabolic Syndrome Eating Plan**. This plan is based on eating less starchy food, with more protein (meat/meat products & fish) and monounsaturated fats found in olive oil, rapeseed oil, avocado, nuts etc.

This eating plan has been **personally designed** for you to be **lower in calories** than what you need, and you should lose at least 1/2kg or 1Ib per week.

It is very important that you stick to the plan as closely as possible and if you experience difficulties to speak your study monitor.

Your name: _____

Your study monitor is:_____

Telephone: _____

Eating Plan

Starchy Foods
Servings per day

A Serving is:

- 3 tbsp. breakfast cereal / muesli
- 3 tbsp. dry porridge oats*
- 1 slice of bread or toast (granary is best)*

- 2 crispbreads
- ¼ baguette (granary is best)*
- 2 ryvita's* or crisp breads

2 rice cakes
½ bread roll/ bagel
½ pita or 1 mini pita*

- ½ English muffin
- ½ plain scone
- ½ English fruit muffin*
- 9 small rice crackers

1 crumpet
½ fruit scone*
1 scotch pancake
3 cream crackers

- 3 heaped tbsp. boiled pasta*
- 2 egg sized boiled potatoes (75g)
- ½ cup boiled noodles*
- 1/4 plain naan bread

1/2 chapatti*
2 heaped tbsp. boiled rice
1 small corn cob or ½ cup*
3 papadums (microwaved)

- 3 cups plain popcorn
- ½ tortilla bread*
- 3 heaped tbsp cooked couscous*

½ cup cooked parsnip
¼ large tin spaghetti (100g)

*** Slow acting starch.**

Starchy Foods – Fast or Slow Acting?

Some starchy food digests slowly which means your blood sugar levels rise slowly and gradually. Other starch food causes a faster rise in your blood sugar because it digests quicker. The SLOW ACTING foods are better because they fill you up for longer and help to reduce the amount of insulin your body makes.

Try to have **at LEAST ONE SLOW ACTING food each day**
These are example of slow acting foods
Breakfast cereal:
Porriage oats, all bran, Special K, Just right,
Muesli with dried fruit (check fat is less than 10g per 100g)

Breads:
Granary breads and rolls containing visible seeds.
Pita bread, ryvita's, chapatti, English muffin with dried fruit, Scone with dried fruit

Pasta:
All varieties
Rice:
Basmati rice
Noodles:
All varieties

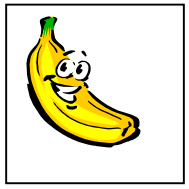
Starchy vegetables:
Sweet potato, sweet corn

Fruit

Servings per day

A serving is:

- 1 medium size piece of fresh fruit
- 2-3 small pieces of fruit
- 1 handful of grapes (or ½ cup)
- 1 small banana, ½ large banana
- 1 ½ cups of melon such as cantaloupe or water melon
- 1 ½ cups of berries such as strawberries, raspberries.
- 1 cup of cherries
- 2 tablespoons fruit (stewed or tinned in juice)
- a small glass of fruit juice (125 ml)
- 1 tablespoon of dried fruit (3 dates, 4 figs).
- 3 cups of raw rhubarb.



Vegetables

Servings per day

A serving is:

- 3 tablespoons of fresh or frozen vegetables (excluding potatoes, corn, parsnips or yam, see starchy foods)
- 1 cup of leafy vegetables (eg lettuce, spinach)
- 1 tomato, 4-5 cherry tomatoes

Milk and Yoghurt:

Servings per day

A serving is:

- 1/2 pint (200ml or 1 glass) of semi-skimmed or skimmed milk
- 1 small pot of low fat no added sugar yoghurt/ fromage frais (eg muller light, shape).
- Check total carbohydrate for the yoghurt is less than 10g per 100g.

Protein Foods:

Servings per day

A serving is:

- 30g (1 oz) raw lean beef, pork, lamb, mince, chicken, turkey or fish (canned or fresh)
- 1 thin slices of lean cold meat.
- 1 egg (limit to 5 eggs per week)
- 1 match box size piece of hard cheese (30g/1 oz).
- 60g (2 oz) of cottage or ricotta cheese
- 60g (2oz) of low fat soft cheese.
- 60g (2oz) of tofu
- 60g (2oz) of quorn

- Choose lean cuts of meat and trim all fat off meat before cooking.
- Aim to have **oily fish twice a week** (canned or fresh salmon, makeral, sardines, herring, trout, pilchards). Choose canned fish in brine, water, or tomato sauce
- Have **beef or lamb no more than twice a week**
- Limit **hard cheese to 3-4 oz per week**

Pulses and Lentils:
These foods contain both protein and starch. You need to count these as part of your protein and starch allowance.

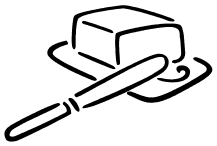
- ½ cup of kidney beans, lentils, chick peas and other pulses = 1 protein & 1 starchy serving.
- 1 small tin baked beans (200g) = 1 protein & 2 starchy servings

Fats and Oils:

Servings per day

A serving is:

- 1 teaspoon of margarine
- 1 teaspoon oil (olive or rapeseed)
- 2 teaspoons of peanut butter
- 1 teaspoon mayonnaise or salad cream
- 2 teaspoons low fat spread / low fat dressing
- 1 tablespoon cream
- ⅛ avocado
- 3 teaspoons of pesto
- 1 teaspoon tahini



- Try to use a margarine high in monounsaturated fats such as olive oil spreads eg olivio,, butterley, clover, I can believe its not butter.
- Use **olive oil or rapeseed oil** in your cooking.
- Limit cream to less than once a week.

Drinks

- Tea and coffee can be drunk freely, but remember to count the milk in the milk allowance. If you have sugar in your tea or coffee try using an artificial sweetener such as canderal or hermesetas
- Water, sugar free squashes and diet or sugar free fizzy drinks can be drunk freely.
- Count any fruit juice (even 100% pure) as part of your fruit allowance.

Alcohol

If you drink alcohol, aim to limit to no more than 2 units per day for women and 3 units for men. You should also try to have 2 alcohol free days each week.

You will need to count any alcoholic drinks as part of your portion plan:

	Fat Portion	Starch Portion
1 pint beer or lager	2	1
Small glass wine (125 ml)	2	0
1 nip of spirits (pub measure)	2	0

Free Foods (Unlimited) :

- water
- tea or coffee (with milk from allowance)
- sugar free squashes and sugar free fizzy drinks
- Marmite, Oxo, Bovril, Vegemite
- stock cubes, herbs and spices

Cooking Sauces and Ready Meals :

Look at how much carbohydrate, fat and protein is contained in the portion of food **you will eat**. This will usually be the whole meal for a ready meal or ¼ jar of cooking sauce.

15g carbohydrate = 1 starch portion
7g protein = 1 protein portion
5g fat = 1 fat & oils portion

For example: Ready made lasagne (1/2 pack):
32g carbohydrate = 2 starch portions
20g protein = 3 protein portions
13g fat = 3 fat and oil portion

½ frozen pizza:
40g carbohydrate = 3 starch portions
20.5g protein = 3 protein portions
14.4g fat = 3 fat portions

Miscellanous Foods and Snacks:

Some foods contain a combination of starch/sugar and fat. For these foods you will need to count these as part of your eating plan. Try to have these foods occasionally (1-2 times a week) and count as part of your portion plan.

	Fat Portion	Starch Portion
Crisps (1 small bag, 25g)	1	1
2 Plain biscuit (ie digestive, rich tea)	1	1
1 Chocolate biscuit, shortbread	1	1
Chocolate bar (50g)	3	2
Fun size chocolate bar (eg two finger kit kat, milky way	1	1
sweet muffin (eg chocolate)	3	2
Fruit tart	1	2
Small piece of plain cake	1	2
Croissant	1	3
Do nought	2	2
Apple pie (1/6 of pie)	3	3
Cheese cake (1/6 of cake)	2	3
Ice cream (one scoop)	1	1
Jam / marmalade (3 tsp)	0	1
Low calorie hot chocolate	0	1/2
Tomato based pasta sauce	0	1
Stir in tomato pasta sauce	1	1/2
Stir in creamy pasta sauce	2	0
Nuts	3	1 protein

- ***Daily Eating Plan***
- You have been allocated specific number of servings of each of the food groups. Here is a sample meal plan for you:

• Food group	• Example
• **Breakfast** • ___ Starchy • ___ Milk & yoghurt • ___ Fats & Oils • ___ Fruit • ___ Protein	•
• Mid Morning •	•
• **Lunch** • ___ Starchy • ___ Protein • ___ Fats & Oils • ___ Fruit • ___ Vegetable • ___ Milk & yoghurt •	•
• Mid Afternoon •	•
• **Dinner** • ___ Starchy • ___ Protein • ___ Fats & Oils • ___ Fruit • ___ Vegetable • ___ Milk & yoghurt	•

Notes

Figure 48: Copy of the eating plan given to those on a HC diet

Healthy Eating Plan

You have been selected to participate in this study as you have a number of risk factors for disease such as high blood pressure, cholesterol, larger than ideal waist measure, and higher sugar levels.

You have been randomly allocated to follow the **Healthy Eating Plan**. This plan is based on eating plenty of starchy foods, moderate amounts of protein foods and less fatty foods.

This eating plan has been **personally designed** for you to be **lower in calories** than what you need, and you should lose at least 1/2kg or 1Ib per week.

It is very important that you stick to the plan as closely as Possible and if you experience difficulties to speak your study monitor.

Your name: _____

Your study monitor is:_____

Telephone: _____

Starchy Foods

Servings per day
A Serving is:

- 3 tbsp. breakfast cereal / muesli
- 3 tbsp. dry porridge oats
- 1 slice of bread or toast

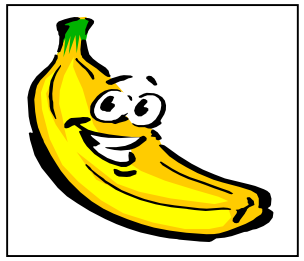
• 2 crispbreads	2 rice cakes
• ¼ baguette	½ bread roll/ bagel
• 2 ryvita's or crisp breads	½ pita or 1 mini pita
• ½ English muffin	1 crumpet
• ½ plain scone	½ fruit scone
• ½ English fruit muffin	1 scotch pancake
• 9 small rice crackers	3 cream crackers
• 3 heaped tbsp. boiled pasta	1/2 chapatti
• 2 egg sized boiled potatoes (75g)	2 heaped tbsp. boiled rice
• ½ cup boiled noodles	1 small corn cob or ½ cup
• 1/4 plain naan bread	3 papadums (microwaved)
• 3 cups plain popcorn	½ cup cooked parsnip
• ½ tortilla bread (100g)	¼ large tin spaghetti
• 3 heaped tbsp cooked couscous	

Fruit

Servings of fruit per day

A serving is:

- 1 medium size piece of fresh fruit
- 2-3 small pieces of fruit
- 1 handful of grapes (or ½ cup)
- 1 small banana, ½ large banana
- 1 ½ cups of melon such as cantaloupe or water melon
- 1 ½ cups of berries such as strawberries, raspberries.
- 1 cup of cherries
- 2 tablespoons fruit (stewed or tinned in juice)
- a small glass of fruit juice (125 ml)
- 1 tablespoon of dried fruit (3 dates, 4 figs).
- 3 cups of raw rhubarb.



Protein Foods:

Servings per day

A serving is:

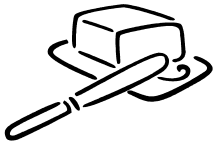
- 30g (1 oz) raw lean beef, pork, lamb, mince, chicken, turkey or fish (canned or fresh)
- 1 thin slices of lean cold meat.
- 1 egg (limit to 5 eggs per week)
- 1 match box size piece of hard cheese (30g/1 oz).
- 60g (2oz) of cottage or ricotta cheese
- 30g (1oz) of low fat soft cheese.
- 60g (2oz) of tofu
- 60g (2oz) of quorn

- Choose lean cuts of meat and trim all fat off meat before cooking.
- Have **beef or lamb no more than twice a week**
- Limit **hard cheese to 3-4 oz per week**

Pulses and Lentils:
These foods contain both protein and starch. You need to count these as part of your protein and starch allowance.

- ½ cup of kidney beans, lentils, chick peas and other pulses = 1 protein & 1 starchy serving.
- 1 small tin baked beans (200g) = 1 protein & 2 starchy servings

Fats and Oils:



Servings per day

A serving is:
- 1 teaspoon margarine
- 1 teaspoon oil
- 2 teaspoons of peanut butter
- 1 teaspoon mayonnaise or salad cream
- 2 teaspoons low fat spread / low fat dressing
- 1 tablespoon cream
- ⅛ avocado,
- 1 teaspoon tahini
- 3 teaspoons pesto

- Limit cream to less than once a week.

Vegetables

Servings of vegetables per day

A serving is:
- 3 tablespoons of fresh or frozen vegetables (excluding potatoes, peas, corn, parsnips or yam, see starchy foods)

- 1 cup of leafy vegetables (eg lettuce, spinach)
- 1 tomato, 4-5 cherry tomatoes

Milk and Yoghurt:

Servings per day

A serving is:
- 1/2 pint (200ml or 1 glass) of semi-skimmed or skimmed milk
- 1 small pot of low fat no added sugar yoghurt/ fromage frais (eg muller light, shape).
- Check total carbohydrate for the yoghurt is less than 10g per 100g.

Drinks

- Tea and coffee can be drunk freely, but remember to
- count the milk in the milk allowance. If you have sugar in your tea or coffee try using an artificial sweetener such
- as canderal or hermesetas
- Water, sugar free squashes and diet or sugar free fizzy drinks can be drunk freely.
- Count any fruit juice (even 100% pure) as part of your fruit allowance.

Alcohol

If you drink alcohol, aim to limit to no more than 2 units per day for women and 3 units for men. You should also try to have 2 alcohol free days each week.

You will need to count any alcoholic drinks as part of your portion plan:

	Fat Portion	Starch Portion
1 pint beer or lager	2	1
Small glass wine (125 ml)	2	0
1 nip of spirits (pub measure)	2	0

Free Foods (Unlimited) :

- water
- tea or coffee (with milk from allowance)
- sugar free squashes and sugar free fizzy drinks
- Marmite, Oxo, Bovril, Vegemite
- stock cubes, herbs and spices

Cooking Sauces and Ready Meals :

Look at how much carbohydrate, fat and protein is contained in the portion of food **you will eat.** This will usually be the whole meal for a ready meal or ¼ jar of cooking sauce.

15g carbohydrate = 1 starch portion
7g protein = 1 protein portion
5g fat = 1 fat & oils portion

For example: Ready made lasagne (1/2 pack):
32g carbohydrate = 2 starch portions
20g protein = 3 protein portions
13g fat = 3 fat and oil portion

½ frozen pizza:
40g carbohydrate = 3 starch portions
20.5g protein = 3 protein portions
14.4g fat = 3 fat portions

Miscellanous Foods and Snacks:

Some foods contain a combination of starch/sugar. For these foods you will need to count these as part of your eating plan. Try to have these foods occasionally (1-2 times a week) and count as part of your portion plan.

	Fat Portion	Starch Portion
Crisps (1 small bag, 25g)	1	1
2 Plain biscuit (ie digestive, rich tea)	1	1
1 Chocolate biscuit, shortbread	1	1
Chocolate bar (50g)	3	2
Fun size chocolate bar (eg two finger kit kat, milky way	1	1
sweet muffin (eg chocolate)	3	2
Fruit tart	1	2
Small piece of plain cake	1	2
Croissant	1	3
Do nought	2	2
Apple pie (1/6 of pie)	3	3
Cheese cake (1/6 of cake)	2	3
Ice cream (one scoop)	1	1
Jam / marmalade (3 tsp)	0	1
Low calorie hot chocolate	0	1/2
Tomato based pasta sauce	0	1
Stir in tomato pasta sauce	1	1/2
Stir in creamy pasta sauce	2	0
Nuts (30g, 1oz)	3	1 protein

Daily Eating Plan

You have been allocated specific number of servings of each of the food groups. Here is a sample meal plan for you

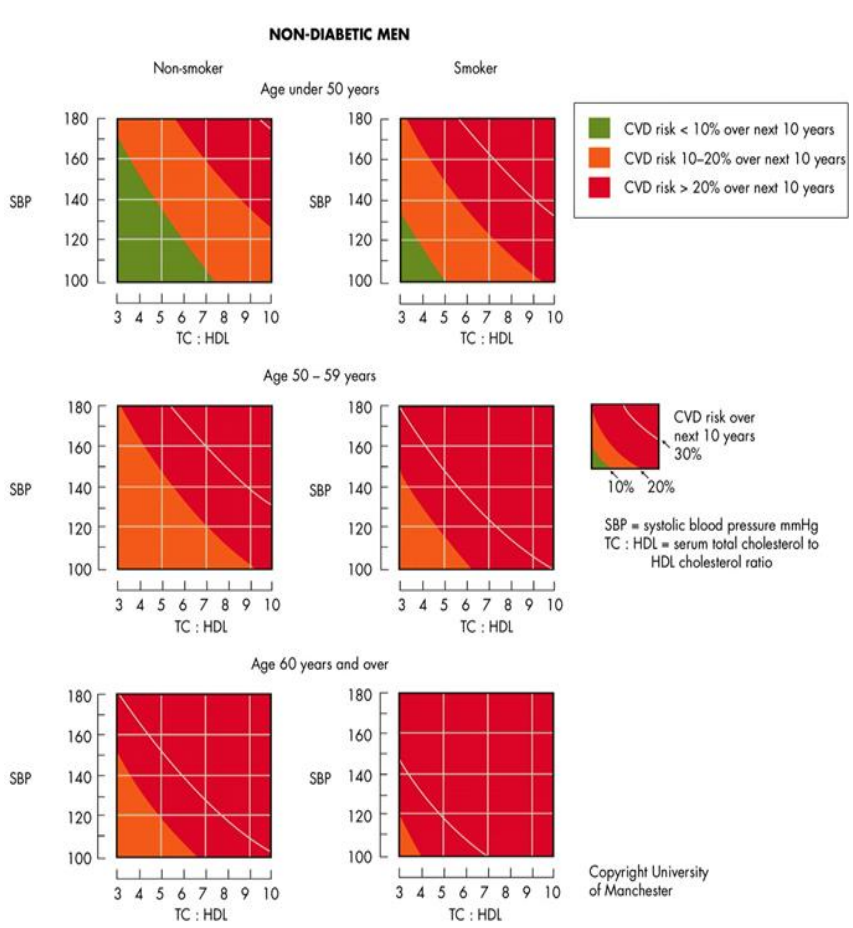
Food group	Example
Breakfast ___ Starchy ___ Milk & yoghurt ___ Fats & Oils ___ Fruit ___ Protein	
Mid Morning	
Midday meal ___ Starchy ___ Protein ___ Fats & Oils ___ Fruit ___ Vegetable ___ Milk & yoghurt	
Mid Afternoon	
Evening Meal ___ Starchy ___ Protein ___ Fats & Oils ___ Fruit ___ Vegetable ___ Milk & yoghurt	

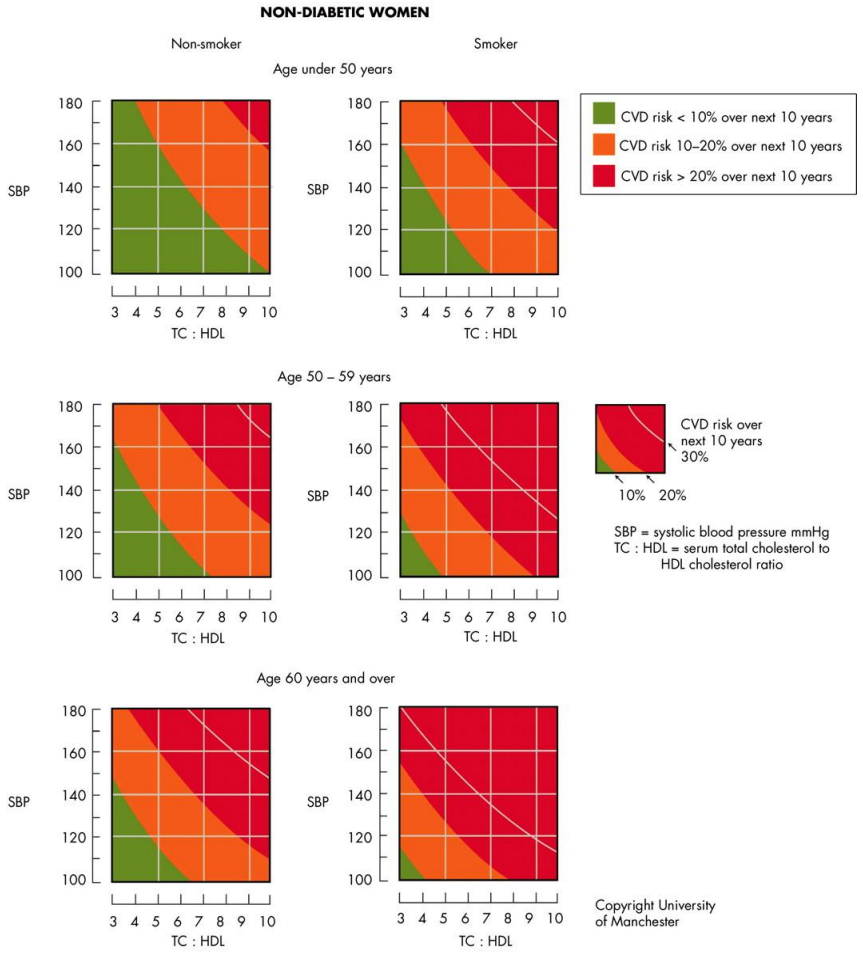
Table 57: Visit Crib Sheet

Plan of action at each visit

Week	Consent	Physical exam	Waist Circum	Weight	B/P	Bloods	GTT	Food Diary	Diet counsel
-4	Y	Y							
-2			Y	Y	Y	Y	Y	Y(7)	Y
0			Y	Y	Y				Y
2				Y					Y
4				Y					Y
6				Y	Y			Y(4)	Y
8				Y					Y
10				Y					Y
12			Y	Y	Y	Y		Y(3)	Y
Month									
4				Y					Y
5				Y					Y
6		Y	Y	Y	Y	Y	Y	Y(3)	Y
7				Y					Y
8				Y					Y
9			Y	Y	Y				Y
10				Y					Y
11				Y					Y
12		Y	Y	Y	Y	Y	Y	Y(3)	Y

Figure 49: Joint British Societies cardiovascular risk prediction charts for men and women without diabetes[579]





Reference List

(1) Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ* 1998; 317(7160):703-713.

(2) Evaluation of the Counterweight Programme for obesity management in primary care: a starting point for continuous improvement. *Br J Gen Pract* 2008; 58(553):548-554.

(3) Percent of Obese (BMI > 30) in U.S. Adults. 2012. 2013.
Ref Type: Online Source

(4) Statistics on obesity, physical activity and diet: England, 2012. 2012. The Health and Social Care Information Centre. 2013.
Ref Type: Online Source

(5) Venus von Willendorf. 2012. 2011.
Ref Type: Online Source

(6) A Report of the Panel on Macronutrients SoURLoNaIaUoDRIatSCotSEoDRI. Dietary References Intakes for Energy, Carbohydrate, Fiber, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients). The National Academies Press; 2005.

(7) Abbasi F, Carantoni M, Chen Y-DI, Reaven GM. Further evidence for a central role of adipose tissue in the antihyperglycemic effect of metformin. *Diabetes Care* 1998; 21(8):1301-1305.

(8) Abbasi F, Lamendola C, McLaughlin T, Hayden J, Reaven GM, Reaven PD. Plasma adiponectin concentrations do not increase in association with moderate weight loss in insulin-resistant, obese women. *Metabolism* 2004; 53(3):280-283.

(9) Agatston A. The South Beach Diet: A Doctor's Plan for Fast and Lasting Weight Loss . Headline; 2005.

(10) Agueda M, Lasa A, Simon E, Ares R, Larrarte E, Labayen I. Association of circulating visfatin concentrations with insulin resistance and low-grade inflammation after dietary energy restriction in Spanish obese non-diabetic women: Role of body composition changes. *Nutrition, Metabolism and Cardiovascular Diseases* 2012; 22(3):208-214.

(11) Ahmed SB, Fisher ND, Stevanovic R, Hollenberg NK. Body mass index and angiotensin-dependent control of the renal circulation in healthy humans. *Hypertension* 2005; 46(6):1316-1320.

(12) Al-Daghri N, Bartlett WA, Jones F, Kumar S. Role of leptin in glucose metabolism in type 2 diabetes. *Diabetes Obes Metab* 2002; 4(3):147-155.

(13) Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; 366(9491):1059-1062.

(14) Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; 15(7):539-553.

(15) Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA et al. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; 120(16):1640-1645.

(16) Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: Possible link between visceral fat accumulation and vascular disease. *Diabetes* 1997; 46(5):860-867.

(17) Alexander CM, Landsman PB, Grundy SM. The influence of age and body mass index on the metabolic syndrome and its components. *Diabetes Obes Metab* 2008; 10(3):246-250.

(18) Alexander CM, Landsman PB, Teutsch SM, Haffner SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 2003; 52(5):1210-1214.

(19) Andreotti F, Davies GJ, Hackett D, Khan MI, De Bart A, Dooijewaard G et al. Circadian variation of fibrinolytic factors in normal human plasma. *Fibrinolysis* 1988; 2(SUPPL. 2):90-92.

(20) Annonymous. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. UK Prospective Diabetes Study Group. *BMJ* 1998; 317(7160):713-720.

(21) Annonymous. National Collaborating Centre for Chronic Conditions. Type 2 diabetes: national clinical guideline for management in primary and secondary care (update). National Collaborating Centre for Chronic Conditions, editor. 2008. London, Royal College of Physicians. 11-11-2013.
Ref Type: Online Source

(22) Annonymous. The European Medicines Agency recommends suspension of the marketing authorisation of Acomplia. European Medicines Agency. In press 2008.

(23) Annonymous. European Medicines Agency recommends suspension of marketing authorisation for sibutramine. European Medicines Agency. In press 2010.

(24) Annonymous. Adult Obesity Prevalence within England. National Obesity Observatory, editor. 2012. 2012.
Ref Type: Online Source

(25) Anonymous. Erratum: Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34) (The Lancet (1998) (854)). *Lancet* 1998; 352(9139):1558.

(26) Anonymous. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285(19):2486-2497.

(27) Anonymous. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: A randomised placebo-controlled trial. *Lancet* 2003; 361(9374):2002-2016.

(28) Appel SJ, Harrell JS, Davenport ML. Central obesity, the metabolic syndrome, and plasminogen activator inhibitor-1 in young adults. *J Am Acad Nurse Pract* 2005; 17(12):535-541.

(29) Araki S, Dobashi K, Kubo K, Kawagoe R, Yamamoto Y, Kawada Y et al. Plasma Visfatin Concentration as a Surrogate Marker for Visceral Fat Accumulation in Obese Children. *Obesity* 2008; 16(2):384-388.

(30) Aravanis C, Corcondilas A, Dontas AS, Lekos D, Keys A. Coronary heart disease in seven countries. IX. The Greek islands of Crete and Corfu. *Circulation* 1970; 41(4 Suppl):I88-100.

(31) Arita Y, Kihara S, Ouchi N, Takahashi H, Maeda K, Miyagawa J-I et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257(1):79-83.

(32) Armitage J, Collins R, Bowman L, Parish S, Sleight P, Peto R et al. The effects of cholesterol lowering with simvastatin on cause-specific mortality and on cancer incidence in 20,536 high-risk people: A randomised placebo-controlled trial [ISRCTN48489393]. *BMC Medicine* 2005; 3:21.

(33) Arner P. Editorial: Visfatin - A true or false trail to type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91(1):28-30.

(34) Aronne LJ, Brown WV, Isoldi KK. Cardiovascular disease in obesity: A review of related risk factors and risk-reduction strategies. *J Clin Lipidol* 2007; 1(6):575-582.

(35) ASCEND Clinical Trial Service Unit. ASCEND A Study of Cardiovascular Events iN Diabetes. 2013.
Ref Type: Online Source

(36) Assmann G, Schulte H. The Prospective Cardiovascular Munster (PROCAM) study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. *Am Heart J* 1988; 116(6 Pt 2):1713-1724.

(37) Astrup A. Dietary fat and obesity: Still an important issue. *Scand J Nutr* 2003; 47(2):50-57.

(38) Astrup A. The role of dietary fat in obesity. *Semin Vasc Med* 2005; 5(1):40-47.

(39) Astrup A, Finer N. Redefining Type 2 diabetes: 'Diabesity' or 'Obesity Dependent Diabetes Mellitus'? *Obes Rev* 2000; 1(2):57-59.

(40) Atkins R.C. Dr Atkins New Diet Revolution. Vermillion; 2003.

(41) Aucott L, Rothnie H, McIntyre L, Thapa M, Waweru C, Gray D. Long-Term Weight Loss From Lifestyle Intervention Benefits Blood Pressure?: A Systematic Review. *Hypertension* 2009; 54(4):756-762.

(42) Austin MA, King M-C, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990; 82(2):495-506.

(43) Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi T, Azizi F. Beneficial effects of a dietary approaches to stop hypertension eating plan on features of the metabolic syndrome. *Diabetes Care* 2005; 28(12):2823-2831.

(44) Bacon SL, Sherwood A, Hinderliter A, Blumenthal JA. Effects of exercise, diet and weight loss on high blood pressure. *Sports Med* 2004; 34(5):307-316.

(45) Bailey CJ. Metformin Revisited: Its Actions and Indications for Use. *Diabetic Medicine* 1988; 5(4):315-320.

(46) Bailey CJ, Day C. Metformin: its botanical background. *Pract Diab Int* 2004; 21(3):115-117.

(47) Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V, George D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: Association with subclinical inflammation. *J Clin Endocrinol Metab* 2007; 92(5):1971-1974.

(48) Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2004; 109(7):837-842.

(49) Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999; 84(1):137-144.

(50) Banting W. Letter on corpulence, addressed to the public. 1869. *Obes Res* 1993; 1(2):153-163.

(51) Barclay AW, Flood VM, Brand-Miller JC, Mitchell P. Validity of carbohydrate, glycaemic index and glycaemic load data obtained using a semi-quantitative food-frequency questionnaire. *Public Health Nutr* 2008; 11(6):573-580.

(52) Barter PJ, Ballantyne CM, Carmena R, Cabezas MC, Chapman MJ, COUTURE P et al. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. *Journal of Internal Medicine* 2006; 259(3):247-258.

(53) Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007; 357(21):2109-2122.

(54) Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. *Circ Res* 2004; 95(8):764-772.

(55) Baskin ML, Ard J, Franklin F, Allison DB. Prevalence of obesity in the United States. *Obes Rev* 2005; 6(1):5-7.

(56) Bavenholm P, de FU, Landou C, Efendic S, Nilsson J, Wiman B et al. Progression of coronary artery disease in young male post-infarction patients is linked to disturbances of carbohydrate and lipoprotein metabolism and to impaired fibrinolytic function. *Eur Heart J* 1998; 19(3):402-410.

(57) Belalcazar LM, Ballantyne CM, Lang W, Haffner SM, Rushing J, Schwenke DC et al. Metabolic factors, adipose tissue, and plasminogen activator inhibitor-1 levels in type 2 diabetes: findings from the look AHEAD study. *Arterioscler Thromb Vasc Biol* 2011; 31(7):1689-1695.

(58) Belalcazar LM, Lang W, Haffner SM, Hoogeveen RC, Pi-Sunyer FX, Schwenke DC et al. Adiponectin and the mediation of HDL-cholesterol change with improved lifestyle: the Look AHEAD Study. *J Lipid Res* 2012; 53(12):2726-2733.

(59) Belch J, MacCuish A, Campbell I, Cobbe S, Taylor R, Prescott R et al. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. *BMJ* 2008; 337:a1840.

(60) Bell SJ, Sears B. Low-glycemic-load diets: Impact on obesity and chronic diseases. *Crit Rev Food Sci Nutr* 2003; 43(3):357-377.

(61) Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR et al. Plasma Visfatin Concentrations and Fat DepotΓÇôSpecific mRNA Expression in Humans. *Diabetes* 2005; 54(10):2911-2916.

(62) Bjerre Knudsen L, Madsen LW, Andersen S+, Almholt K, de Boer AS, Drucker DJ et al. Glucagon-Like Peptide-1 Receptor Agonists Activate Rodent Thyroid C-Cells Causing Calcitonin Release and C-Cell Proliferation. *Endocrinology* 2010; 151(4):1473-1486.

(63) Black PH. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun* 2003; 17(5):350-364.

(64) Blumenthal JA BMH. Effects of the dash diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and women with high blood pressure: The encore study. *Arch Intern Med* 2010; 170(2):126-135.

(65) Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G et al. Prevalence of insulin resistance in metabolic disorders: The Bruneck Study. *Diabetes* 1998; 47(10):1643-1649.

(66) Botella-Carretero J, Luque-Ramrez M, Alvarez-Blasco F, Peromingo R, Millín JL, Escobar-Morreale H. The Increase in Serum Visfatin After Bariatric Surgery in Morbidly Obese Women is Modulated by Weight Loss, Waist Circumference, and Presence or Absence of Diabetes Before Surgery. *OBES SURG* 2008; 18(8):1000-1006.

(67) Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the management of diabetes: A meta-analysis of randomized controlled trials. *Diabetes Care* 2003; 26(8):2261-2267.

(68) Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD et al. Efficacy and safety of low-carbohydrate diets: A systematic review. *JAMA* 2003; 289(14):1837-1850.

(69) Bray GA. Low-carbohydrate diets and realities of weight loss. *JAMA* 2003; 289(14):1853-1855.

(70) Bray GA, Paeratakul S, Popkin BM. Dietary fat and obesity: a review of animal, clinical and epidemiological studies. *Physiol Behav* 2004; 83(4):549-555.

(71) Bray GA, Tartaglia LA. Medicinal strategies in the treatment of obesity. *Nature* 2000; 404(6778):672-677.

(72) Brehm BJ, Lattin BL, Summer SS, Boback JA, Gilchrist GM, Jandacek RJ et al. One-Year Comparison of a High Monounsaturated Fat Diet With a High-Carbohydrate Diet in Type 2 Diabetes. *Diabetes Care* 2009; 32(2):215-220.

(73) Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab* 2003; 88(4):1617-1623.

(74) Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K et al. Cardiovascular Events Associated with Rofecoxib in a Colorectal Adenoma Chemoprevention Trial. *New England Journal of Medicine* 2005; 352(11):1092-1102.

(75) Britton KA, Fox CS. Ectopic Fat Depots and Cardiovascular Disease. *Circulation* 2011; 124(24):e837-e841.

(76) Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E et al. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res* 2000; 8(9):605-619.

(77) Brown JEP, Onyango DJ, Ramanjaneya M, Conner AC, Patel ST, Dunmore SJ et al. Visfatin regulates insulin secretion, insulin receptor signalling and mRNA expression of diabetes-related genes in mouse pancreatic +¦-cells. *Journal of Molecular Endocrinology* 2010; 44(3):171-178.

(78) Brown PJ, Konner M. An anthropological perspective on obesity. *Ann N Y Acad Sci* 1987; 499:29-46.

(79) Burkitt DP, Trowell HC. Dietary fibre and western diseases. *Ir Med J* 1977; 70(9):272-277.

(80) Burri BJ, Neidlinger TR, Van Loan M, Keim NL. Effect of low-calorie diets on plasma retinol-binding protein concentrations in overweight women. *J Nutr Biochem* 1990; 1(9):484-486.

(81) Buse JB, Nauck M, Forst T, Sheu WH, Shenouda SK, Heilmann CR et al. Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study. *Lancet* 2013; 381(9861):117-124.

(82) Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. Effects of Exenatide (Exendin-4) on Glycemic Control Over 30 Weeks in Sulfonylurea-Treated Patients With Type 2 Diabetes. *Diabetes Care* 2004; 27(11):2628-2635.

(83) Caballero B. The global epidemic of obesity: An overview. *Epidemiol Rev* 2007; 29(1):1-5.

(84) Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. *Cardiovasc Res* 2000; 87(10):840-844.

(85) Campbell IW, Menzies DG, Chalmers J, McBain AM, Brown IR. One year comparative trial of metformin and glipizide in type 2 diabetes mellitus. *Diabete Metab* 1994; 20(4):394-400.

(86) Campbell RK, White JR, Jr., Saulie BA. Metformin: a new oral biguanide. *Clin Ther* 1996; 18(3):360-371.

(87) Cardillo S, Seshadri P, Iqbal N. The effects of low-carbohydrate versus low-fat diet on adipocytokines in severely obese adult: Three-year follow-up of a randomized trial. *Eur Rev Med Pharmacol Sci* 2006; 10(3):99-106.

(88) Case CC, Jones PH, Nelson K, O'Brian Smith E, Ballantyne CM. Impact of weight loss on the metabolic syndrome. *Diabetes Obes Metab* 2002; 4(6):407-414.

(89) Cassady BA, Charboneau NL, Brys EE, Crouse KA, Beitz DC, Wilson T. Effects of low carbohydrate diets high in red meats or poultry, fish and shellfish on plasma lipids and weight loss. *Nutr Metab* 2007; 4.

(90) Chait JR, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoprotein to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 1993; 94(4):350-356.

(91) Chan DC, Watts GF, Ng TWK, Uchida Y, Sakai N, Yamashita S et al. Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Atherosclerosis Supplements 6[1], 32. 2005.
Ref Type: Abstract

(92) Chen C-C, Li T-C, Li C-I, Liu C-S, Lin W-Y, Wu M-T et al. The relationship between visfatin levels and anthropometric and metabolic parameters: association with cholesterol levels in women. *Metabolism* 2007; 56(9):1216-1220.

(93) Chen M-P, Chung F-M, Chang D-M, Tsai JCR, Huang H-F, Shin S-J et al. Elevated plasma level of visfatin/pre-β cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91(1):295-299.

(94) Cho YM, Youn B-S, Lee H, Lee N, Min S-S, Kwak SH et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 2006; 29(11):2457-2461.

(95) Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report. *JAMA* 2003; 289(19):2560-2572.

(96) Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *The Lancet* 2013; 376(9753):1670-1681.

(97) Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet* 2007; 370(9600):1706-1713.

(98) Cleeman JI. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 2001; 285(19):2486-2497.

(99) Clifton PM, Noakes M, Nestel PJ. LDL particle size and LDL and HDL cholesterol changes with dietary fat and cholesterol in healthy subjects. *J Lipid Res* 1998; 39(9):1799-1804.

(100) Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HAW, Livingstone SJ et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): Multicentre randomised placebo-controlled trial. *Lancet* 2004; 364(9435):685-696.

(101) Collins R, Armitage J, Parish S, Sleight P, Peto R. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20 536 high-risk individuals: A randomised placebo-controlled trial. *Lancet* 2002; 360(9326):7-22.

(102) Consultation WE. Waist Circumference and Waist-Hip Ratio. 2008. Geneva. Ref Type: Pamphlet

(103) Coughlin S, Calverley P, Wilding J. Sleep disordered breathing--a new component of syndrome x? *Obes Rev* 2001; 2(4):267-274.

(104) Coulston AM, Hollenbeck CB, Swislocki AL, Reaven GM. Persistence of hypertriglyceridemic effect of low-fat high-carbohydrate diets in NIDDM patients. *Diabetes Care* 1989; 12(2):94-101.

(105) Coulston AM, Liu GC, Reaven GM. Plasma glucose, insulin and lipid responses to high-carbohydrate low-fat diets in normal humans. *Metabolism* 1983; 32(1):52-56.

(106) Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A et al. C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. *New England Journal of Medicine* 2004; 350(14):1387-1397.

(107) Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S et al. Overweight in children and adolescents: Pathophysiology, consequences, prevention, and treatment. *Circulation* 2005; 111(15):1999-2012.

(108) Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *The American Journal of Clinical Nutrition* 1992; 56(2):320-328.

(109) De Courten BV, Degawa-Yamauchi M, Considine RV, Tataranni PA. High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. *Diabetes* 2004; 53(5):1279-1284.

(110) De Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008; 582(1):97-105.

(111) De Luis DA, Gonzalez SM, Conde R, Aller R, Izaola O, Castro MJ et al. Lack of effect of a moderate hypocaloric diet on visfatin levels in morbid obese patients: relationship with insulin resistance. *Eur Rev Med Pharmacol Sci* 2010; 14(12):1031-1036.

(112) De Luis DA, Gonzalez SM, Conde R, Aller R, Izaola O, Perez Castrillon JL et al. Relation of resistin levels with cardiovascular risk factors and insulin resistance in non-diabetes obese patients. *Diabetes Res Clin Pract* 2009; 84(2):174-178.

(113) De Luis DA, Gonzalez SM, Conde R, Aller R, Izaola O, Romero E. Effect of a hypocaloric diet on serum visfatin in obese non-diabetic patients. *Nutrition* 2008; 24(6):517-521.

(114) De Luis DA, Izaola O, Conde R, Primo D, Sagrado MG, Aller R. Visfatin levels in female, morbid, nondiabetic obese patients after biliopancreatic diversion surgery. *Surg Obes Relat Dis* 2011; 7(2):195-198.

(115) De KL, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Eur Heart J* 2007; 28(7):850-856.

(116) DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991; 14(3):173-194.

(117) DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of Exenatide (Exendin-4) on Glycemic Control and Weight Over 30 Weeks in Metformin-Treated Patients With Type 2 Diabetes. *Diabetes Care* 2005; 28(5):1092-1100.

(118) Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R et al. Serum Resistin (FIZZ3) Protein Is Increased in Obese Humans. *J Clin Endocrinol Metab* 2003; 88(11):5452-5455.

(119) Dekker JM, Funahashi T, Nijpels G, Pilz S, Stehouwer CD, Snijder MB et al. Prognostic value of adiponectin for cardiovascular disease and mortality. *J Clin Endocrinol Metab* 2008; 93(4):1489-1496.

(120) Del Rincon I, Williams K, Stern MP, Freeman GL, Escalante An. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis & Rheumatism* 2001; 44(12):2737-2745.

(121) Denker PS, Pollock VE. Fasting serum insulin levels in essential hypertension. A meta-analysis. *Arch Intern Med* 1992; 152(8):1649-1651.

(122) Denova-Gutierrez E, Huitron-Bravo G, Talavera JO, Castanon S, Gallegos-Carrillo K, Flores Y et al. Dietary glycemic index, dietary glycemic load, blood lipids, and coronary heart disease. *J Nutr Metab* 2010; 2010.

(123) Department of Health. National Diet and Nutrition Survey: Headline resultsfrom Years 1 and 2 (combined) of the rolling programme 2009/9 - 2009/10. Crown; 2011.

(124) Diabetes Trials Unit. HOMA Calculator v2.2. Oxford Centre for Diabetes, Endocrinology & Metabolism . 30-6-2004.
Ref Type: Internet Communication

(125) Didangelos TP, Thanopoulou AK, Bousboulas SH, Sambanis CL, Athyros VG, Spanou EA et al. The ORLIstat and CArdiovascular risk profile in patients with metabolic syndrome and type 2 DIAbetes (ORLICARDIA) study. *Curr Med Res Opin* 2004; 20(9):1393-1401.

(126) Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial in macroVascular Events): A randomised controlled trial. *Lancet* 2005; 366(9493):1279-1289.

(127) Doyle ME, Egan JM. Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacol Ther* 2007; 113(3):546-593.

(128) Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proceedings of the National Academy of Sciences* 1987; 84(10):3434-3438.

(129) Dumesnil JG, Turgeon J, Tremblay A, Poirier P, Gilbert M, Gagnon L et al. Effect of a low-glycaemic index--low-fat--high protein diet on the atherogenic metabolic risk profile of abdominally obese men. *Br J Nutr* 2001; 86(5):557-568.

(130) Dunstan DW, Zimmet PZ, Welborn TA, de Court, Cameron AJ, Sicree RA et al. The Rising Prevalence of Diabetes and Impaired Glucose Tolerance. *Diabetes Care* 2002; 25(5):829-834.

(131) Eades MR, Eades MD. Protein Power: The High-Protein/Low-Carbohydrate Way to Lose Weight, Feel Fit, and Boost Your H ealth--in Just Weeks! Random House Publishing Group; 2009.

(132) Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a Low-Glycemic Load vs Low-Fat Diet in Obese Young Adults: A Randomized Trial. *JAMA* 2007; 297(19):2092-2102.

(133) Ebbeling CB, Leidig MM, Sinclair KB, Seger-Shippee LG, Feldman HA, Ludwig DS. Effects of an *ad libitum* low-glycemic load diet on cardiovascular disease risk factors in obese young adults. *Am J Clin Nutr* 2005; 81(5):976-982.

(134) Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; 365(9468):1415-1428.

(135) Eckel RH, Yost TJ, Jensen DR. Alterations in lipoprotein lipase in insulin resistance. *Int J Obes Relat Metab Disord* 1995; 19 Suppl 1:S16-S21.

(136) Erdem G, Dogru T, Tasci I, Bozoglu E, Muhsiroglu O, Tapan S et al. The effects of pioglitazone and metformin on plasma visfatin levels in patients with treatment naive type 2 diabetes mellitus. Diabetes Research and Clinical Practice 82[2], 214-218. 1-11-2008.
Ref Type: Abstract

(137) Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003-2006. *Natl Health Stat Report* 2009;(13):1-7.

(138) Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: A randomized trial. *JAMA* 2004; 292(12):1440-1446.

(139) Estruch R, Martinez-Gonzelez MAl, orella D, alas-Salvado J, uiz-Gutieürrez V, ovas MI et al. Effects of a Mediterranean-Style Diet on Cardiovascular Risk FactorsA Randomized Trial. *Annals of Internal Medicine* 2006; 145(1):1-11.

(140) European Medicines Agency. European Medicines Agency recommends new contra-indications and warnings for pioglitazone to reduce small increased risk of bladder cancer: benefit-risk balance remains positive in a limited population of type 2 diabetics. 21-7-2011. 2012.
Ref Type: Online Source

(141) European Medicines Agency. European Medicines Agency confirms recommendation to suspend Tredaptive, Pelzont and Trevaclyn. 2013. 2013.
Ref Type: Online Source

(142) Ewbank PP, Darga LL, Lucas CP. Physical activity as a predictor of weight maintenance in previously obese subjects. *Obes Res* 1995; 3(3):257-263.

(143) Fargnoli JL, Fung TT, Olenczuk DM, Chamberland JP, Hu FB, Mantzoros CS. Adherence to healthy eating patterns is associated with higher circulating total and high-molecular-weight adiponectin and lower resistin concentrations in women from the Nurses' Health Study. *Am J Clin Nutr* 2008; 88(5):1213-1224.

(144) Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr* 2003; 78(1):31-39.

(145) Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 1999; 341(12):879-884.

(146) Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002; 110(8):1093-1103.

(147) Farooqi IS, O'Rahilly S. Genetics of obesity in humans. *Endocr Rev* 2006; 27(7):710-718.

(148) Feinglos MN, Saad MF, Pi-Sunyer FX, An B, Santiago O, on behalf of the Liraglutide Dose-Response Study Group. Effects of liraglutide (NN2211), a long-acting GLP-1 analogue, on glycaemic control and bodyweight in subjects with Type-á2 diabetes. *Diabetic Medicine* 2005; 22(8):1016-1023.

(149) Feldeisen SE, Tucker KL. Nutritional strategies in the prevention and treatment of metabolic syndrome. *Appl Physiol Nutr Metab* 2007; 32(1):46-60.

(150) Fenton C, Chee CM, Bergqvist AGC. Manipulation of Types of Fats and Cholesterol Intake Can Successfully Improve the Lipid Profile While Maintaining the Efficacy of the Ketogenic Diet. *ICAN: Infant, Child, & Adolescent Nutrition* 2009; 1(6):338-341.

(151) Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jarvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR). *Hypertension* 1997; 30(5):1144-1149.

(152) Ferroni P, Basili S, Falco A, Davì G. Inflammation, insulin resistance, and obesity. *Curr Atheroscler Rep* 2004; 6(6):424-431.

(153) Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: The Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 2000; 102(1):42-47.

(154) Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. *Circulation* 2006; 113(14):1753-1759.

(155) Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation* 2000; 102(9):1000-1006.

(156) Fichtlscherer S, Zeiher AM. Endothelial dysfunction in acute coronary syndromes: association with elevated C-reactive protein levels. *Ann Med* 2000; 32(8):515-518.

(157) Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates. *Health Aff* 2009; 28(5):w822-w831.

(158) Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. *JAMA* 2005; 293(15):1861-1867.

(159) Food Standards Agency. FSA Nutrient and Food Based Guidelines for UK Institutions. 2006. Food Standards Agency.
Ref Type: Report

(160) Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA : The Journal of the American Medical Association [NLM - MEDLINE]* 2002; 287(3):356.

(161) Ford ES, Liu S. Glycemic index and serum high-density lipoprotein cholesterol concentration among us adults. *Arch Intern Med* 2001; 161(4):572-576.

(162) Forsythe CE, Phinney SD, Fernandez ML, Quann EE, Wood RJ, Bibus DM et al. Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids* 2008; 43(1):65-77.

(163) Fosbol EL, Folke F, Jacobsen S+, Rasmussen JN, S+©rensen R, Schramm TK et al. Cause-Specific Cardiovascular Risk Associated With Nonsteroidal Antiinflammatory Drugs Among Healthy Individuals. *Circulation: Cardiovascular Quality and Outcomes* 2010.

(164) Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003; 348(21):2082-2090.

(165) Foster-Powell K, Holt SHA, Brand-Miller JC. International table of gylcemic index and glycemic load values: 2002. *Am J Clin Nutr* 2002; 76(1):5-56.

(166) Fragopoulou E, Panagiotakos DB, Pitsavos C, Tampourlou M, Chrysohoou C, Nomikos T et al. The association between adherence to the Mediterranean diet and adiponectin levels among healthy adults: the ATTICA study. *The Journal of Nutritional Biochemistry* 2010; 21(4):285-289.

(167) Franks PW, Brage S, Luan J, Ekelund U, Rahman M, Farooqi IS et al. Leptin Predicts a Worsening of the Features of the Metabolic Syndrome Independently of Obesity. *Obesity Research* 2005; 13(8):1476-1484.

(168) Frey S, Nagl B, Henze A, Raila J, Schlosser B, Berg T et al. Isoforms of Retinol binding protein 4 (RBP4) are increased in chronic diseases of the kidney but not of the liver. *Lipids in Health and Disease* 2008; 7(1):29.

(169) Fuehrlein BS, Rutenberg MS, Silver JN, Warren MW, Theriaque DW, Duncan GE et al. Differential metabolic effects of saturated versus polyunsaturated fats in ketogenic diets. *J Clin Endocrinol Metab* 2004; 89(4):1641-1645.

(170) Fuenmayor N, Moreira E, Cubeddu LX. Salt sensitivity is associated with insulin resistance in essential hypertension. *Am J Hypertens* 1998; 11(4 Pt 1):397-402.

(171) Fujinami A, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H et al. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. *Clin Chim Acta* 2004; 339(1-2):57-63.

(172) Fujita H, Fujishima H, Morii T, Koshimura J, Narita T, Kakei M et al. Effect of metformin on adipose tissue resistin expression in db/db mice. *Biochem Biophys Res Commun* 2002; 298(3):345-349.

(173) Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K et al. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005; 307(5708):426-430.

(174) Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K et al. Retraction. *Science* 2007; 318(5850):565.

(175) Fukui Y, Motojima K. Expression of resistin in the adipose tissue is modulated by various factors including peroxisome proliferator-activated receptor α. *Diabetes Obes Metab* 2002; 4(5):342-345.

(176) Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; 114(12):1752-1761.

(177) Gannon MC, Nuttall FQ. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes* 2004; 53(9):2375-2382.

(178) Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. *Am J Clin Nutr* 2003; 78(4):734-741.

(179) Gannon MC, Nuttall JA, Damberg G, Gupta V, Nuttall FQ. Effect of protein ingestion on the glucose appearance rate in people with type 2 diabetes. *J Clin Endocrinol Metab* 2001; 86(3):1040-1047.

(180) Gannon MC, Nuttall JA, Nuttall FQ. The metabolic response to ingested glycine. *Am J Clin Nutr* 2002; 76(6):1302-1307.

(181) Garaulet M, Hernández-Morante JJ, De Heredia FP, Tébar FJ. Adiponectin, the controversial hormone. *Public Health Nutr* 2007; 10(10 A):1145-1150.

(182) Garcia-Fuentes E, Garcia-Almeida JM, Garcia-Arnos J, Garcia-Serrano S, Rivas-Maran J, Gallego-Perales JL et al. Plasma Visfatin Concentrations in Severely Obese Subjects Are Increased After Intestinal Bypass. *Obesity* 2007; 15(10):2391-2395.

(183) Gardner CD, Kraemer HC. Monounsaturated Versus Polyunsaturated Dietary Fat and Serum Lipids: A Meta-analysis. *Arterioscler Thromb Vasc Biol* 1995; 15(11):1917-1927.

(184) Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: A meta-analysis. *Am J Clin Nutr* 1998; 67(3 Suppl):577S-582S.

(185) Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK et al. Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *JAMA* 1994; 271(18):1421-1428.

(186) Garten A, Petzold S, Barnikol-Oettler A, Korner A, Thasler WE, Kratzsch J et al. Nicotinamide phosphoribosyltransferase (NAMPT/PBEF/visfatin) is constitutively released from human hepatocytes. *Biochem Biophys Res Commun* 2010; 391(1):376-381.

(187) Gerber M, Boettner A, Seidel B, Lammert A, Bar J, Schuster E et al. Serum resistin levels of obese and lean children and adolescents: Biochemical analysis and clinical relevance. *J Clin Endocrinol Metab* 2005; 90(8):4503-4509.

(188) Gil-Campos M, Ca±ete Rn, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clinical Nutrition* 2004; 23(5):963-974.

(189) Ginsberg HN, Elam MB, Lovato LC, Crouse JR, III, Leiter LA, Linz P et al. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 2010; 362(17):1563-1574.

(190) Gnagnarella P, Gandini S, La VC, Maisonneuve P. Glycemic index, glycemic load, and cancer risk: a meta-analysis. *Am J Clin Nutr* 2008; 87(6):1793-1801.

(191) Goldstein DJ. Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord* 1992; 16(6):397-415.

(192) Goodpaster BH DJOAea. Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults: A randomized trial. *JAMA* 2010; 304(16):1795-1802.

(193) Gordon LB, Harten IA, Patti ME, Lichtenstein AH. Reduced adiponectin and HDL cholesterol without elevated C-reactive protein: Clues to the biology of premature atherosclerosis in Hutchinson-Gilford Progeria Syndrome. *The Journal of Pediatrics* 2005; 146(3):336-341.

(194) Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R et al. European guidelines on cardiovascular disease prevention in clinical practice: Executive summary - Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2007; 28(19):2375-2414.

(195) Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 2006; 354(24):2552-2563.

(196) Green BD, Gault VA, O'Harte FP, Flatt PR. Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. *Curr Pharm Des* 2004; 10(29):3651-3662.

(197) Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ et al. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: Relative contribution of small dense LDL to coronary heart disease risk. *Atherosclerosis* 1994; 106(2):241-253.

(198) Grundy SM. Small LDL, atherogenic dyslipidemia, and the metabolic syndrome. *Circulation* 1997; 95(1):1-4.

(199) Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, for the Conference Participants. Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Arterioscler Thromb Vasc Biol* 2004; 24(2):13-18.

(200) Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement: Executive Summary. *Circulation* 2005; 112(17):285-290.

(201) Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2004; 112(17):2735-2752.

(202) Guan H, Wang P, Hui R, Edin ML, Zeldin DC, Wang DW. Adeno-associated virus-mediated human C-reactive protein gene delivery causes endothelial dysfunction and hypertension in rats. *Clin Chem* 2009; 55(2):274-284.

(203) Gupta SK. Intention-to-treat concept: A review. *Perspectives in clinical research* 2011; 2(3):109.

(204) Haffner SM, D'Agostino R, Saad MF, Rewers M, Mykkanen L, Selby J et al. Increased insulin resistance and insulin secretion in nondiabetic African-Americans and Hispanics compared with non-Hispanic whites. The Insulin Resistance Atherosclerosis Study. *Diabetes* 1996; 45(6):742-748.

(205) Haffner SM, Taegtmeyer H. Epidemic obesity and the metabolic syndrome. *Circulation* 2003; 108(13):1541-1545.

(206) Haider DG, Holzer G, Schaller G, Weghuber D, Widhalm K, Wagner O et al. The adipokine visfatin is markedly elevated in obese children. *J Pediatr Gastroenterol Nutr* 2006; 43(4):548-549.

(207) Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia* 2006; 49(8):1909-1914.

(208) Haider DG, Schindler K, Mittermayer F, Muller M, Nowotny P, Rieger A et al. Effect of rosiglitazone on visfatin and retinol-binding protein-4 plasma concentrations in HIV-positive patients. *Clin Pharmacol Ther* 2007; 81(4):580-585.

(209) Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. *J Clin Endocrinol Metab* 2007; 92(3):1168-1171.

(210) Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *Journal of Clinical Endocrinology and Metabolism* 2006; 91(4):1578-1581.

(211) Halle M, Berg A, Baumstark W, K+Ânig D, Huonker M, Keul J. Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels. *Atherosclerosis* 1999; 143(1):185-192.

(212) Halton TL, Hu FB. The Effects of High Protein Diets on Thermogenesis, Satiety and Weight Loss: A Critical Review. *Journal of the American College of Nutrition* 2004; 23(5):373-385.

(213) Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L et al. Effect of weight loss with lifestyle intervention on risk of diabetes. *Diabetes Care* 2006; 29(9):2102-2107.

(214) Hammarstedt A, Pihlajamäki J, Sopasakis VR, Gogg S, Jansson P-A, Laakso M et al. Visfatin is an adipokine, but it is not regulated by thiazolidinediones. *J Clin Endocrinol Metab* 2006; 91(3):1181-1184.

(215) Hamsten A, Walldius G, Szamosi A, Blombäck M, de Faire U, Dahlén G et al. Plasminogen activator inhibitor in plasma: Risk factor for recurrent myocardial infarction. *Lancet* 1987; 330(8549):3-9.

(216) Hansen JB, Grimsgaard S, Nordoy A, Bonaa KH. Dietary Supplementation with Highly Purified Eicosapentaenoic Acid and Docosahexaenoic Acid Does Not Influence PAI-1 Activity. *Thrombosis Research* 2000; 98(2):123-132.

(217) Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet* 1998; 351(9118):1755-1762.

(218) Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care* 2006; 29(6):1357-1362.

(219) Hardie LJ, Rayner DV, Holmes S, Trayhurn P. Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. *Biochem Biophys Res Commun* 1996; 223(3):660-665.

(220) Harlan WR, Landis JR, Flegal KM, Davis CS, Miller ME. Secular trends in body mass in the United States, 1960-1980. *Am J Epidemiol* 1988; 128(5):1065-1074.

(221) Harper A, Astrup A. Can we advise our obese patients to follow the Atkins diet? *Obes Rev* 2004; 5(2):93-94.

(222) Harper CR, Jacobson TA. Beyond the Mediterranean Diet: The role of omega-3 fatty acids in the prevention of coronary heart disease. *Prev Cardiol* 2003; 6(3):136-146.

(223) Harris JA, Benedict FG. A biometric study of human basal metabolism. *Proceedings of the National Academy of Sciences of the United States of America* 1918; 4(12):370.

(224) Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation* 2007; 116(9):1081-1093.

(225) Haslam D. Obesity: a medical history. *Obes Rev* 2007; 8 Suppl 1:31-36.

(226) Haugen F, Jorgensen A, Drevon CA, Trayhurn P. Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. *FEBS Lett* 2001; 507(1):105-108.

(227) Hauptman J, Lucas C, Boldrin MN, Collins H, Segal KR. Orlistat in the long-term treatment of obesity in primary care settings. *Arch Fam Med* 2000; 9(2):160-167.

(228) He FJ, Macgregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens* 2002; 16(11):761-770.

(229) Heilbronn LK, Noakes M, Clifton PM. The Effect of High- and Low-Glycemic Index Energy Restricted Diets on Plasma Lipid and Glucose Profiles in Type 2 Diabetic Subjects with Varying Glycemic Control. *Journal of the American College of Nutrition* 2002; 21(2):120-127.

(230) Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 2004; 89(4):1844-1848.

(231) Heller RF, Heller RF. The carbohydrate addict's diet: the lifelong solution to yo-yo dieting. Dutton; 1991.

(232) Hellerstein MK. Carbohydrate-induced hypertriglyceridemia: modifying factors and implications for cardiovascular risk. *Curr Opin Lipidol* 2002; 13(1):33-40.

(233) Henry CJK. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutrition* 2005; 8(7a):1133-1152.

(234) Henze A, Frey SK, Raila J, Tepel M, Scholze A, Pfeiffer AFH et al. Evidence That Kidney Function but Not Type 2 Diabetes Determines Retinol-Binding Protein 4 Serum Levels. *Diabetes* 2008; 57(12):3323-3326.

(235) Heritier SR, Gebski VJ, Keech AC. Inclusion of patients in clinical trial analysis: the intention-to-treat principle. *Medical Journal of Australia* 2003; 179(8):438-440.

(236) Hernandez-Morante JJ, Milagro F, Gabaldon JA, Martinez JA, Zamora S, Garaulet M. Effect of DHEA-sulfate on adiponectin gene expression in adipose tissue from different fat depots in morbidly obese humans. *Eur J Endocrinol* 2006; 155(4):593-600.

(237) Hession M, Rolland C, Kulkarni U, Wise A, Broom J. Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obesity Reviews* 2009; 10(1):36-50.

(238) Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 1999; 282(16):1568-1575.

(239) Hirsso PK, Timonen MJ, Jokelainen JJ, Hiltunen LA, Laakso MA, Hedberg PSM et al. Association Between High-Sensitive Measurement of C-Reactive Protein and Metabolic Syndrome as Defined by International Diabetes Federation, National Cholesterol Education Program, and World Health Organization Criteria in a Population-Based Cohort of 55-Year-Old Finnish Individuals. *Diabetes Care* 2006; 29(9):2177-2178.

(240) Holst JJ, Vilsboll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. *Molecular and Cellular Endocrinology* 2009; 297(1ΓÇô2):127-136.

(241) Home PD, Pocock SJ, Beck-Nielsen H, Curtis PS, Gomis R, Hanefeld M et al. Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. *Lancet* 2009; 373(9681):2125-2135.

(242) Howard BV, Manson JE, Stefanick ML, Beresford SA, Frank G, Jones B et al. Low-Fat Dietary Pattern and Weight Change Over 7 Years: The Women's Health Initiative Dietary Modification Trial. *JAMA* 2006; 295(1):39-49.

(243) Hsieh C-H, He C-T, Lee C-H, Wu L-Y, Hung Y-J. Both slow-release and regular-form metformin improve glycemic control without altering plasma visfatin level in patients with type 2 diabetes mellitus. *Metabolism* 2007; 56(8):1087-1092.

(244) Hsieh C-J, Wang P-W, Liu R-T, Tung S-C, Chien WY, Chen J-F et al. Orlistat for obesity: Benefits beyond weight loss. *Diabetes Res Clin Pract* 2005; 67(1):78-83.

(245) Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 2001; 345(11):790-797.

(246) Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA et al. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997; 337(21):1491-1499.

(247) Hu W-L, Qiao S-B, Hou Q, Yuan J-S. Plasma resistin is increased in patients with unstable angina. *Chinese Medical Journal* 2007; 120(10):871-875.

(248) Hug C, Lodish HF. Visfatin: A new adipokine. *Science* 2005; 307(5708):366-367.

(249) Inadera H. The usefulness of circulating adipokine levels for the assessment of obesity-related health problems. *Int J Med Sci* 2008; 5(5):248-262.

(250) Ingram DD, Mussolino ME. Weight loss from maximum body weight and mortality: the Third National Health and Nutrition Examination Survey Linked Mortality File. *Int J Obes* 2010; 34(6):1044-1050.

(251) International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome: Part 1: Worldwide definition for use in clinical practice. 2005.
Ref Type: Internet Communication

(252) Iqbal N, Seshadri P, Stern L, Loh J, Kundu S, Jafar T et al. Serum resistin is not associated with obesity or insulin resistance in humans. *Eur Rev Med Pharmacol Sci* 2005; 9(3):161-165.

(253) Jacobs DR, Sluik D, Rokling-Andersen MH, Anderssen SA, Drevon CA. Association of 1-y changes in diet pattern with cardiovascular disease risk factors and adipokines: results from the 1-y randomized Oslo Diet and Exercise Study. *The American Journal of Clinical Nutrition* 2009; 89(2):509-517.

(254) Jain SK, Nagi DK, Slavin BM, Lumb PJ, Yudkin JS. Insulin Therapy in Type 2 Diabetic Subjects Suppresses Plasminogen Activator Inhibitor (PAI-1) Activity and Proinsulin-like Molecules Independently of Glycaemic Control. *Diabetic Medicine* 1993; 10(1):27-32.

(255) Jakicic JM, Egan CM, Fabricatore AN, Gaussoin SA, Glasser SP, Hesson LA et al. Four-Year Change in Cardiorespiratory Fitness and Influence on Glycemic Control in Adults With Type 2 Diabetes in a Randomized Trial: The Look AHEAD Trial. *Diabetes Care* 2012.

(256) Janke J, Engeli S, Boschmann M, Adams F, Böhnke J, Luft FC et al. Retinol-binding protein 4 in human obesity. *Diabetes* 2006; 55(10):2805-2810.

(257) Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin Gene Expression in Human Adipocytes Is Not Related to Insulin Resistance. *Obesity* 2002; 10(1):1-5.

(258) Jarvi AE, Karlstrom BE, Granfeldt YE, Bjorck IE, Asp NG, Vessby BO. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* 1999; 22(1):10-18.

(259) Järvi AE, Karlstrom BE, Granfeldt YE, Björck IE, Asp N-G, Messby BOH. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* 1999; 22(1):10-18.

(260) Jellinger PS. Metabolic consequences of hyperglycemia and insulin resistance. *Clin Cornerstone* 2007; 8(SUPPL. 7):S30-S42.

(261) Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW et al. American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis. *Endocr Pract* 2012; 18 Suppl 1:1-78.

(262) Jendle J, Nauck MA, Matthews DR, Frid A, Hermansen K, D++ring M et al. Weight loss with liraglutide, a once-daily human glucagon-like peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue. *Diabetes, Obesity and Metabolism* 2009; 11(12):1163-1172.

(263) Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 1981; 34(3):362-366.

(264) Jenkins DJ, Kendall CW, Augustin LS, Franceschi S, Hamidi M, Marchie A et al. Glycemic index: overview of implications in health and disease. *Am J Clin Nutr* 2002; 76(1):266S-273.

(265) Jeppesen J, Hansen TW, Rasmussen S, Ibsen H, Torp-Pedersen C. Metabolic syndrome, low-density lipoprotein cholesterol, and risk of cardiovascular disease: a population-based study. *Atherosclerosis* 2006; 189(2):369-374.

(266) Ji CY, Cheng TO. Prevalence and geographic distribution of childhood obesity in China in 2005. *International Journal of Cardiology* 2008; 131(1):1-8.

(267) Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *The American Journal of Clinical Nutrition* 2008; 87(1):44-55.

(268) Jolliffe D. Extent of overweight among US children and adolescents from 1971 to 2000. *Int J Obes Relat Metab Disord* 2003; 28(1):4-9.

(269) Jorgensen ME, Bjerregaard P, Gyntelberg F, Borch-Johnsen K. Prevalence of the metabolic syndrome among the Inuit in Greenland. A comparison between two proposed definitions. *Diabet Med* 2004; 21(11):1237-1242.

(270) Joslin EP. A Diabetic Manual for the Mutual Use of Doctor and Patient. 5th ed. Philadelphia: Lea & Febiger; 1934.

(271) Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; 116(7):1784-1792.

(272) Kahn R, Buse J, Ferrannini E, Stern M. The Metabolic Syndrome: Time for a Critical Appraisal. *Diabetes Care* 2005; 28(9):2289-2304.

(273) Kaplan NM. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med* 1989; 149(7):1514-1520.

(274) Kastorini CM, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The Effect of Mediterranean Diet on Metabolic Syndrome and its Components: A Meta-Analysis of 50 Studies and 534,906 Individuals. *Journal of the American College of Cardiology* 2011; 57(11):1299-1313.

(275) Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation* 2006; 113(1):20-29.

(276) Kay JP, Alemzadeh R, Langley G, D'Angelo L, Smith P, Holshouser S. Beneficial effects of metformin in normoglycemic morbidly obese adolescents. *Metabolism* 2001; 50(12):1457-1461.

(277) Keaney JF, Jr., Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D et al. Obesity and Systemic Oxidative Stress: Clinical Correlates of Oxidative Stress in The Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; 23(3):434-439.

(278) Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; 366(9500):1849-1861.

(279) Kettaneh A, Heude B, Oppert JM, Scherer P, Meyre D, Borys JM et al. Serum adiponectin is related to plasma high-density lipoprotein cholesterol but not to plasma insulin-concentration in healthy children: the FLVS II study. *Metabolism* 2006; 55(9):1171-1176.

(280) Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R et al. The diet and 15-year death rate in the seven countries study. *Am J Epidemiol* 1986; 124(6):903-915.

(281) Kinlay S, Schwartz GG, Olsson AG, Rifai N, Leslie SJ, Sasiela WJ et al. High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. *Circulation* 2003; 108(13):1560-1566.

(282) Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation* 2005; 112(12):1756-1762.

(283) Kleiner K. Meats, no shoots, no leaves. *New Sci* 2004; 182(2449):50-51.

(284) Klimcakova E, Kovacikova M, Stich V, Langin D. Adipokines and dietary interventions in human obesity. *Obes Rev* 2010.

(285) Knight EL, Stampfer MJ, Hankinson SE, Spiegelman D, Curhan GC. The Impact of Protein Intake on Renal Function Decline in Women with Normal Renal Function or Mild Renal Insufficiency. *Annals of Internal Medicine* 2003; 138(6):460-467.

(286) Koebnick C, Wagner K, Garcia AL, Gruendel S, Lahmann PH, Weickert MO et al. Increase in serum resistin during weight loss in overweight subjects is related to lipid metabolism. *Int J Obes* 2006; 30(7):1097-1103.

(287) Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. *Circulation* 2008; 117(25):3238-3249.

(288) Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med* 2000; 342(24):1792-1801.

(289) Kohrt WM, Landt M, Birge SJ, Jr. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. *J Clin Endocrinol Metab* 1996; 81(11):3980-3985.

(290) Kolovou GD, Anagnostopoulou KK, Cokkinos DV. Pathophysiology of dyslipidaemia in the metabolic syndrome. *Postgrad Med J* 2005; 81(956):358-366.

(291) Kolovou GDM, Anagnostopoulou KKB, Salpea KDB, Mikhailidis DPM. The Prevalence of Metabolic Syndrome in Various Populations. [Review]. *American Journal of the Medical Sciences* 2007; 333(6):362-371.

(292) Kona V, Lean M, McCombie L, Morrison D, Counterweight Project Team. Weight-loss maintenance is predicted by higher BMI and greater initial loss in the Counterweight Programme. Obes Rev. 12[supplement 1]. 16-5-2011.
Ref Type: Abstract

(293) Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M et al. Association of adiponectin mutation with type 2 diabetes : A candidate gene for the insulin resistance syndrome. *Diabetes* 2002; 51(7):2325-2328.

(294) Kotani K, Yamada T, Taniguchi N. The association between adiponectin, HDL-cholesterol and alpha1-antitrypsin-LDL in female subjects without metabolic syndrome. *Lipids in Health and Disease* 2010; 9(1):147.

(295) Krebs J, Elley C, Parry-Strong A, Lunt H, Drury P, Bell D et al. The Diabetes Excess Weight Loss (DEWL) Trial: a randomised controlled trial of high-protein versus high-carbohydrate diets over 2-áyears in type 2 diabetes. *Diabetologia*:1-10.

(296) Kruger J, Blanck H, Gillespie C. Dietary and physical activity behaviors among adults successful at weight loss maintenance. *International Journal of Behavioral Nutrition and Physical Activity* 2006; 3(1):17.

(297) Ku CY, Gower BA, Nagy TR, Goran MI. Relationships between dietary fat, body fat, and serum lipid profile in prepubertal children. *Obes Res* 1998; 6(6):400-407.

(298) Kuhn W, Schmalfeldt B, Reuning U, Pache L, Berger U, Ulm K et al. Prognostic significance of urokinase (uPA) and its inhibitor PAI-1 for survival in advanced ovarian carcinoma stage FIGO IIIc. *Br J Cancer* 1999; 79(11-12):1746-1751.

(299) Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation* 2004; 109(17):2046-2049.

(300) Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003; 23(1):85-89.

(301) Kusminski CM, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. *Clin Sci (Colch)* 2005; 109(3):243-256.

(302) Kwon K, Jung SH, Choi C, Park S-H. Reciprocal association between visceral obesity and adiponectin: In healthy premenopausal women. *Int J Cardiol* 2005; 101(3):385-390.

(303) Lago F, Dieguez C, Gómez-Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. *Cytokine Growth Factor Rev* 2007; 18(3-4):313-325.

(304) Lakka H-M, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002; 288(21):2709-2716.

(305) Lamarche B, Moorjani S, Lupien PJ, Cantin B, Bernard PM, Dagenais GR et al. Apolipoprotein A-I and B Levels and the Risk of Ischemic Heart Disease During a Five-Year Follow-up of Men in the Que-¦bec Cardiovascular Study. *Circulation* 1996; 94(3):273-278.

(306) Larsen PJ. Mechanisms behind GLP-1 induced weight loss. *The British Journal of Diabetes & Vascular Disease* 2008; 8(2 suppl):S34-S41.

(307) Larsen TM, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AFH et al. Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. *New England Journal of Medicine* 2010; 363(22):2102-2113.

(308) Larsson SC, Bergkvist L, Wolk A. Glycemic load, glycemic index and breast cancer risk in a prospective cohort of Swedish women. *Int J Cancer* 2009; 125(1):153-157.

(309) Lawlor DA, Davey SG, Ebrahim S, Thompson C, Sattar N. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. *J Clin Endocrinol Metab* 2005; 90(10):5677-5683.

(310) Layman DK. The role of leucine in weight loss diets and glucose homeostasis. *J Nutr* 2003; 133(1):261S-267S.

(311) Layman DK, Baum JI. Dietary protein impact on glycemic control during weight loss. *J Nutr* 2004; 134(4):968S-973S.

(312) Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C et al. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. *J Nutr* 2003; 133(2):411-417.

(313) Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. *J Nutr* 2003; 133(2):405-410.

(314) Lean MEJ, Reckless JPD, Finer N, McCombie L. Counterweight - counter-cost, counter-loss. *International Journal of Clinical Practice* 2010; 64(6):828-829.

(315) Lee A, Morley JE. Metformin Decreases Food Consumption and Induces Weight Loss in Subjects with Obesity with Type II Non-Insulin-Dependent Diabetes. *Obesity Research* 1998; 6(1):47-53.

(316) Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab* 2003; 88(10):4848-4856.

(317) Leeds AR. Glycemic index and heart disease. *Am J Clin Nutr* 2002; 76(1):286S-289S.

(318) Lettner A, Roden M. Ectopic fat and insulin resistance. *Curr Diab Rep* 2008; 8(3):185-191.

(319) Lev-Ran A. Human obesity: an evolutionary approach to understanding our bulging waistline. *Diabetes Metab Res Rev* 2001; 17(5):347-362.

(320) Levey ASGT, Beck GJ, Caggiula AW, Kusek JW, Hunsicker LG, The Modification of Diet in Renal Dis. Dietary Protein Restriction and the Progression of Chronic Renal Disease. *Journal of the American Society of Nephrology* 1999; 10(11):2426-2439.

(321) Levri KM, Slaymaker E, Last A, Yeh J, Ference J, DeAmico F et al. Metformin as Treatment for Overweight and Obese Adults: A Systematic Review. *The Annals of Family Medicine* 2005; 3(5):457-461.

(322) Li L, Yang G, Li Q, Tang Y, Yang M, Yang H et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Exp Clin Endocrinol Diabetes* 2006; 114(10):544-548.

(323) Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420(6917):868-874.

(324) Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006; 114(1):82-96.

(325) Lieb C. The effects on human beings of a twelve months' exclusive meat diet. *JAMA* 1929;(July 6):20-22.

(326) Liese AD, Schulz M, Fang F, Wolever TMS, D'Agostino RB, Jr., Sparks KC et al. Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 2005; 28(12):2832-2838.

(327) Lim SY, Davidson SM, Paramanathan AJ, Smith CC, Yellon DM, Hausenloy DJ. The novel adipocytokine visfatin exerts direct cardioprotective effects. *Journal of cellular and molecular medicine* 2008; 12(4):1395-1403.

(328) Lindstrom J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemio K et al. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet* 2006; 368(9548):1673-1679.

(329) Lindstrom J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J et al. The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. *Diabetes Care* 2003; 26(12):3230-3236.

(330) LIU G, COULSTON A, HOLLENBECK C, REAVEN G. The Effect of Sucrose Content in High and Low Carbohydrate Diets on Plasma Glucose, Insulin, and Lipid Responses in Hypertriglyceridemic Humans. *Journal of Clinical Endocrinology & Metabolism* 1984; 59(4):636-642.

(331) Liu S, Manson JE, Buring JE, Stampfer MJ, Willett WC, Ridker PM. Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. *Am J Clin Nutr* 2002; 75(3):492-498.

(332) Liu S, Manson JE, Stampfer MJ, Holmes MD, Hu FB, Hankinson SE et al. Dietary glycemic load assessed by food-frequency questionnaire in relation to plasma high-density-lipoprotein cholesterol and fasting plasma triacylglycerols in postmenopausal women. *Am J Clin Nutr* 2001; 73(3):560-566.

(333) Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr* 2000; 71(6):1455-1461.

(334) Liu SW, Qiao SB, Yuan JS, Liu DQ. Association of plasma visfatin levels with inflammation, atherosclerosis and acute coronary syndromes (ACS) in humans. *Clin Endocrinol (Oxf)* 2009; 71(2):202-207.

(335) Long SJ, Jeffcoat AR, Millward DJ. Effect of habitual dietary-protein intake on appetite and satiety. *Appetite* 2000; 35(1):79-88.

(336) Lopez-Bermejo A, Chico-Julia B, Fernandez-Balsells M, Recasens M, Esteve E, Casamitjana R et al. Serum visfatin increases with progressive beta-cell deterioration. *Diabetes* 2006; 55(10):2871-2875.

(337) Lorenzo C, Serrano-Rios M, Martinez-Larrad MT, Gonzalez-Sanchez JL, Seclen S, Villena A et al. Geographic variations of the International Diabetes Federation and the National Cholesterol Education Program-Adult Treatment Panel III definitions of the metabolic syndrome in nondiabetic subjects. *Diabetes Care* 2006; 29(3):685-691.

(338) Ludwig DS. Dietary glycemic index and obesity. *J Nutr* 2000; 130(2 Suppl):280S-283S.

(339) Ludwig DS. Clinical update: The low-glycaemic-index diet. *Lancet* 2007; 369(9565):890-892.

(340) Ma L-J, Mao S-L, Taylor KL, Kanjanabuch T, Guan YF, Zhang YH et al. Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 2004; 53(2):336-346.

(341) Mackarness R. Eat Fat and Grow Slim. 1-1-1958.
Ref Type: Serial (Book,Monograph)

(342) Maebuchi M, Machidori M, Urade R, Ogawa T, Moriyama T. Low resistin levels in adipose tissues and serum in high-fat fed mice and genetically obese mice: Development of an ELISA system for quantification of resistin. *Arch Biochem Biophys* 2003; 416(2):164-170.

(343) Maki KC, Rains TM, Kaden VN, Raneri KR, Davidson MH. Effects of a reduced-glycemic-load diet on body weight, body composition, and cardiovascular disease risk markers in overweight and obese adults. *The American Journal of Clinical Nutrition* 2007; 85(3):724-734.

(344) Makimattila S, Nikkila K, Yki-Jarvinen H. Causes of weight gain during insulin therapy with and without metformin in patients with Type II diabetes mellitus. *Diabetologia* 1999; 42(4):406-412.

(345) Manco M, Fernandez-Real JM, Equitani F, Vendrell J, Mora MEV, Nanni G et al. Effect of massive weight loss on inflammatory adipocytokines and the innate immune system in morbidly obese women. *J Clin Endocrinol Metab* 2007; 92(2):483-490.

(346) Manley SE. U.K. Prospective diabetes study 27: Plasma lipids and lipoproteins at diagnosis of NIDDM by age and sex. *Diabetes Care* 1997; 20(11):1683-1687.

(347) Mantzoros CS, Li T, Manson JE, Meigs JB, Hu FB. Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes. *J Clin Endocrinol Metab* 2005; 90(8):4542-4548.

(348) Marckmann P, Sandstrom B, Jespersen J. Favorable long-term effect of a low-fat/high-fiber diet on human blood coagulation and fibrinolysis. *Arterioscler Thromb Vasc Biol* 1993; 13(4):505-511.

(349) Martin M, Palaniappan LP, Kwan AC, Reaven GM, Reaven PD. Ethnic Differences in the Relationship Between Adiponectin and Insulin Sensitivity in South Asian and Caucasian Women. *Diabetes Care* 2008; 31(4):798-801.

(350) Maruyama C, Ishibashi R, Araki R, Koike S, Hirose H, Maruyama T. HMW-Adiponectin Associates with Triglyceride Concentrations in Type 1 Diabetic Patients. *Journal of Atherosclerosis and Thrombosis* 2009; 16(3):207-216.

(351) Maryniuk MD. The new shape of medical nutrition therapy. *Diabetes Spectrum* 2000; 13(3):122.

(352) Masulli M, Patti L, Riccardi G, Vaccaro O, Annuzzi G, Ebbesson SOE et al. Relation Among Lipoprotein Subfractions and Carotid Atherosclerosis in Alaskan Eskimos (from the GOCADAN Study). The American journal of cardiology 104[11], 1516-1521. 1-12-2009.
Ref Type: Abstract

(353) Mather KJ, Steinberg HO, Baron AD. Weight loss and endothelial function in obesity. *Diabetes Care* 2003; 26(6):1927-1928.

(354) Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* 2002; 147(2):173-180.

(355) Matsubara M, Namioka K, Katayose S. Decreased plasma adiponectin concentrations in women with low-grade C-reactive protein elevation. *Eur J Endocrinol* 2003; 148(6):657-662.

(356) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7):412-419.

(357) Mavri A, Alessi MC, Bastelica D, Geel-Georgelin O, Fina F, Sentocnik JT et al. Subcutaneous abdominal, but not femoral fat expression of plasminogen activator inhibitor-1 (PAI-1) is related to plasma PAI-1 levels and insulin resistance and decreases after weight loss. *Diabetologia* 2001; 44(11):2025-2031.

(358) McAuley KA, Hopkins CM, Smith KJ, McLay RT, Williams SM, Taylor RW et al. Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. *Diabetologia* 2005; 48(1):8-16.

(359) McAuley KA, Smith KJ, Taylor RW, McLay RT, Williams SM, Mann JI. Long-term effects of popular dietary approaches on weight loss and features of insulin resistance. *Int J Obes Relat Metab Disord* 2005; 30(2):342-349.

(360) McLaren L. Socioeconomic status and obesity. *Epidemiol Rev* 2007; 29(1):29-48.

(361) McLaughlin T, Abbasi F, Lamendola C, Liang L, Reaven G, Schaaf P et al. Differentiation between obesity and insulin resistance in the association with C-reactive protein. *Circulation* 2002; 106(23):2908-2912.

(362) McMillan-Price J, Petocz P, Atkinson F, Samman S, Steinbeck K, Caterson I et al. Comparison of 4 diets of varying glycemic load on weight loss and cardiovascular risk reduction in overweight and obese young adults: A randomized controlled trial. *Arch Intern Med* 2006; 166(14):1466-1475.

(363) McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, Leonnetti DL et al. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care* 1999; 22(1):65-70.

(364) McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S. Resistin, central obesity, and type 2 diabetes. *The Lancet* 2002; 359(9300):46-47.

(365) McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN et al. Increased Resistin Gene and Protein Expression in Human Abdominal Adipose Tissue. *J Clin Endocrinol Metab* 2002; 87(5):2407.

(366) Meckling KA, Gauthier M, Grubb R, Sanford J. Effects of a hypocaloric, low-carbohydrate diet on weight loss, blood lipids, blood pressure, glucose tolerance, and body composition in free-living overweight women. *Can J Physiol Pharmacol* 2002; 80(11):1095-1105.

(367) Meckling KA, O'Sullivan C, Saari D. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *J Clin Endocrinol Metab* 2004; 89(6):2717-2723.

(368) Milton JE, Briche B, Brown IJ, Hickson M, Robertson CE, Frost GS. Relationship of glycaemic index with cardiovascular risk factors: analysis of the National Diet and Nutrition Survey for people aged 65 and older. *Public Health Nutr* 2007; 10(11):1321-1335.

(369) Miranda PJ, DeFronzo RA, Califf RM, Guyton JR. Metabolic syndrome: Definition, pathophysiology, and mechanisms. *Am Heart J* 2005; 149(1):33-45.

(370) Misra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition* 2003; 19(5):457-466.

(371) Miyatake N, Matsumoto S, Fujii M, Numata T. Reducing waist circumference by at least 3 cm is recommended for improving metabolic syndrome in obese Japanese men. *Diabetes Res Clin Pract* 2008; 79(2):191-195.

(372) Miyatake N, Matsumoto S, Miyachi M, Fujii M, Numata T. Relationship between changes in body weight and waist circumference in Japanese. *Environ Health Prev Med* 2007; 12(5):220-223.

(373) Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, *in vivo*. *J Clin Endocrinol Metab* 1997; 82(12):4196-4200.

(374) Moore GBT, Chapman H, Holder JC, Lister CA, Piercy V, Smith SA et al. Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice. *Biochem Biophys Res Commun* 2001; 286(4):735-741.

(375) Morange PE, Lijnen HR, Alessi MC, Kopp F, Collen D, Juhan-Vague I. Influence of PAI-1 on adipose tissue growth and metabolic parameters in a murine model of diet-induced obesity. *Arterioscler Thromb Vasc Biol* 2000; 20(4):1150-1154.

(376) Muredach RP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin Is an Inflammatory Marker of Atherosclerosis in Humans. *Circulation* 2005; 111(7):932-939.

(377) Musaad S, Haynes EN. Biomarkers of obesity and subsequent cardiovascular events. *Epidemiol Rev* 2007; 29(1):98-114.

(378) Muzio F, Mondazzi L, Sommariva D, Branchi A. Long-term effects of low-calorie diet on the metabolic syndrome in obese nondiabetic patients. *Diabetes Care* 2005; 28(6):1485-1486.

(379) Naderali EK, Pickavance LC, Wilding JPH, Williams G. Diet-induced endothelial dysfunction in the rat is independent of the degree of increase in total body weight. *Clin Sci (Colch)* 2001; 100(6):635-641.

(380) Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun* 2001; 285(2):561-564.

(381) Nakata M, Yada T, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. *Diabetes* 1999; 48(2):426-429.

(382) National Institute of Health and Clinical Excellence. CG67 Lipid modification: Cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease. 2010. National Institute of Health and Clinical Excellence. 2011.
Ref Type: Online Source

(383) Nauck MA, Duran S, Kim D, Johns D, Northrup J, Festa A et al. A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study. *Diabetologia* 2007; 50(2):259-267.

(384) Nelson L. A Hebrew - English Bible According to the Masoretic Text and the JPS 1917 Edition. Deuterenonomy Chapter 14. [JPS 1917 Edition]. 17-10-2012. 2012.
Ref Type: Online Source

(385) Nesto R. C-reactive protein, its role in inflammation, Type 2 diabetes and cardiovascular disease, and the effects of insulin-sensitizing treatment with thiazolidinediones. *Diabet Med* 2004; 21(8):810-817.

(386) Neter JE, Stam BE, Kok FJ, Grobbee DE, Geleijnse JM. Influence of Weight Reduction on Blood Pressure. *Hypertension* 2003; 42(5):878-884.

(387) Neter JE, Stam BE, Kok FJ, Grobbee DE, Geleijnse JM. Influence of Weight Reduction on Blood Pressure: A Meta-Analysis of Randomized Controlled Trials. *Hypertension* 2003; 42(5):878-884.

(388) Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF et al. Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *The American Journal of Clinical Nutrition* 2004; 79(4):544-551.

(389) Ninomiya JK, Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. Association of the Metabolic Syndrome With History of Myocardial Infarction and Stroke in the Third National Health and Nutrition Examination Survey. *Circulation* 2004; 109(1):42-46.

(390) Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; 356(24):2457-2471.

(391) Noakes M, Foster PR, Keogh JB, James AP, Mamo JC, Clifton PM. Comparison of isocaloric very low carbohydrate/high saturated fat and high carbohydrate/low saturated fat diets on body composition and cardiovascular risk. *Nutr Metab (Lond)* 2006; 3:7.

(392) Norata GD, Ongari M, Garlaschelli K, Raselli S, Grigore L, Catapano AL. Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur J Endocrinol* 2007; 156(2):279-284.

(393) Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS, Jr., Brehm BJ et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: A meta-analysis of randomized controlled trials. *Arch Intern Med* 2006; 166(3):285-293.

(394) NovoNordisk. Interim Report on Comparison of liragluatide versus placebo in weight loss maintenance in obese subjects (SCALE) . 2011.
Ref Type: Online Source

(395) Nuttall FQ, Gannon MC, Wald JL, Ahmed M. Plasma glucose and insulin profiles in normal subjects ingesting diets of varying carbohydrate, fat, and protein content. *J Am Coll Nutr* 1985; 4(4):437-450.

(396) Nuttall FQ, Mooradian AD, Gannon MC, Billington C, Krezowski P. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care* 1984; 7(5):465-470.

(397) Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006; 295(13):1549-1555.

(398) Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P. Adiponectin: A key adipocytokine in metabolic syndrome. *Clin Sci (Colch)* 2006; 110(3):267-278.

(399) Oki K, Yamane K, Kamei N, Nojima H, Kohno N. Circulating visfatin level is correlated with inflammation, but not with insulin resistance. *Clinical Endocrinology* 2007; 67(5):796-800.

(400) Orr JB. Report on A Survey of Adequacy of Diet Food Health and Income in Relation to Income. *Socialist Health Association* 1936.

(401) Orr JB. Diet and Nutrition: Nutrition Problems, Dietary Requirements for Health. *Can Med Assoc J* 1939; 41(1):78-80.

(402) Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 2001; 103(8):1057-1063.

(403) Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 2003; 107(5):671-674.

(404) Ozsahin AK, Gokcel A, Sezgin N, Akbaba M, Guvener N, Ozisik L et al. Prevalence of the metabolic syndrome in a Turkish adult population. *Diabetes Nutr Metab* 2004; 17(4):230-234.

(405) Packard CJ. Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochem Soc Trans* 2003; 31:1066-1069.

(406) Padwal RS, Majumdar SR. Drug treatments for obesity: Orlistat, sibutramine, and rimonabant. *Lancet* 2007; 369(9555):71-77.

(407) Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R et al. Reduced plasma visfatin/pre-β cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab* 2006; 91(8):3165-3170.

(408) Paget J. On the Structure and Physiology of Fat. London Medical Gazette: Or, Journal of Practical Medicine. 1840. 674-678.

(409) Pan X, Li GHY. Effect of dietary and/or exercise intervention on incidence of diabetes in 530 subjects with impaired glucose tolerance from 1986 to 1992. *Chung Hua Nei Ko Tsa Chih* 1995; 34(8):112.

(410) Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care* 2002; 25(3):425-430.

(411) Pasman WJ, Westerterp-Plantenga MS, Saris WH. The effect of exercise training on leptin levels in obese males. *Am J Physiol* 1998; 274(2 Pt 1):E280-E286.

(412) Patel A, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 2007; 370(9590):829-840.

(413) Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP. Measurement Characteristics of the Womens Health Initiative Food Frequency Questionnaire. *Annals of Epidemiology* 1999; 9(3):178-187.

(414) Pedersen TR. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344(8934):1383-1389.

(415) Peino R, Pineiro V, Gualillo O, Menendez C, Brenlla J, Casabiell X et al. Cold exposure inhibits leptin secretion in vitro by a direct and non-specific action on adipose tissue. *Eur J Endocrinol* 2000; 142(2):195-199.

(416) Pelkman CL, Fishell VK, Maddox DH, Pearson TA, Mauger DT, Kris-Etherton PM. Effects of moderate-fat (from monounsaturated fat) and low-fat weight-loss diets on the serum lipid profile in overweight and obese men and women. *Am J Clin Nutr* 2004; 79(2):204-212.

(417) Perez-Jimenez F, Lopez-Miranda J, Pinillos MD, Gomez P, Paz-Rojas E, Montilla P et al. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* 2001; 44(11):2038-2043.

(418) Pfützner A, Hanefeld M, Lübben G, Weber MM, Karaglannis E, Kohler C et al. Visfatin: A putative biomarker for metabolic syndrome is not influenced by pioglitazone or simvastatin treatment in nondiabetic patients at cardiovascular risk - Results from the PIOSTAT study. *Horm Metab Res* 2007; 39(10):764-768.

(419) Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA* 2006; 295(7):761-775.

(420) Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP et al. Fasting plasma leptin, tumor necrosis factor-a receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women impact on cardiovascular mortality. *Diabetes Care* 2003; 26(10):2883-2889.

(421) Pignone M, Alberts MJ, Colwell JA, Cushman M, Inzucchi SE, Mukherjee D et al. Aspirin for primary prevention of cardiovascular events in people with diabetes: a position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation. *Diabetes Care* 2010; 33(6):1395-1402.

(422) Pischon T, Bamberger CM, Kratzsch J, Zyriax BC, Algenstaedt P, Boeing H et al. Association of plasma resistin levels with coronary heart disease in women. *Obes Res* 2005; 13(10):1764-1771.

(423) Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004; 291(14):1730-1737.

(424) Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. *The American Journal of Clinical Nutrition* 2005; 81(4):780-786.

(425) Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab* 2004; 89(2):447-452.

(426) Poobalan A, Aucott L, Smith WC, Avenell A, Jung R, Broom J et al. Effects of weight loss in overweight/obese individuals and long-term lipid outcomes--a systematic review. *Obes Rev* 2004; 5(1):43-50.

(427) Poobalan A, Aucott L, Smith WCS, Avenell A, Jung R, Broom J et al. Effects of weight loss in overweight/obese individuals and long-term lipid outcomes – a systematic review. *Obesity Reviews* 2004; 5(1):43-50.

(428) Poortmans JR, Dellalieux O. Do regular high protein diets have potential health risks on kidney function in athletes? *Int J Sport Nutr Exerc Metab* 2000; 10(1):28-38.

(429) Poppitt SD, Keogh GF, Prentice AM, Williams DEM, Sonnemans HMW, Valk EEJ et al. Long-term effects of *ad libitum* low-fat, high-carbohydrate diets on body weight and serum lipids in overweight subjects with metabolic syndrome. *Am J Clin Nutr* 2002; 75(1):11-20.

(430) Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. *JAMA* 2002; 288(8):980-987.

(431) Prospective SC. Body-mass index and cause-specific mortality in 900,000 adults: collaborative analyses of 57 prospective studies. *The Lancet* 2009; 373(9669):1083-1096.

(432) Qi L, Rimm E, Liu S, Rifai N, Hu FB. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. *Diabetes Care* 2005; 28(5):1022-1028.

(433) Qiao L, Zou C, van der Westhuyzen DR, Shao J. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. *Diabetes* 2008; 57(7):1824-1833.

(434) Quehenberger P, Exner M, Sunder-Plassmann R, Ruzicka K, Bieglmayer C, Endler G et al. Leptin induces endothelin-1 in endothelial cells *in vitro*. *Circ Res* 2002; 90(6):711-718.

(435) Ramos EJB, Xu Y, Romanova I, Middleton F, Chen C, Quinn R et al. Is obesity an inflammatory disease? *Surgery* 2003; 134(2):329-335.

(436) Ramsay L, Williams B, Johnston G, MacGregor G, Poston L, Potter J et al. Guidelines for management of hypertension: report of the third working party of the British Hypertension Society. *J Hum Hypertens* 1999; 13(9):569-592.

(437) Rana JS, Visser ME, Arsenault BJ, Despr+®s JP, Stroes ESG, Kastelein JJP et al. Metabolic dyslipidemia and risk of future coronary heart disease in apparently healthy men and women: The EPIC-Norfolk prospective population study. *International Journal of Cardiology* 2010; 143(3):399-404.

(438) Rasouli N, Kern PA. Adipocytokines and the Metabolic Complications of Obesity. *J Clin Endocrinol Metab* 2008; 93(11_Supplement_1):s64-s73.

(439) Ratner RE, Maggs D, Nielsen LL, Stonehouse AH, Poon T, Zhang B et al. Long-term effects of exenatide therapy over 82 weeks on glycaemic control and weight in over-weight metformin-treated patients with type 2 diabetes mellitus. *Diabetes, Obesity and Metabolism* 2006; 8(4):419-428.

(440) Ray KK SSE. Statins and all-cause mortality in high-risk primary prevention: A meta-analysis of 11 randomized controlled trials involving 65-á229 participants. *Arch Intern Med* 2010; 170(12):1024-1031.

(441) Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988; 37(12):1595-1607.

(442) Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 2005; 111(7):932-939.

(443) Reinehr T, Roth CL, Menke T, Andler W. Resistin concentrations before and after weight loss in obese children. *Int J Obes Relat Metab Disord* 2005; 30(2):297-301.

(444) Reiter A. Weight loss does not lower heart disease risk from type 2 diabetes. 2012. National Institutes of Health . 2012.
Ref Type: Online Source

(445) Rennie KL, Jebb SA. Prevalence of obesity in Great Britain. *Obes Rev* 2005; 6(1):11-12.

(446) Retnakaran R, Youn BS, Liu Y, Hanley AJ, Lee NS, Park JW et al. Correlation of circulating full-length visfatin (PBEF/NAMPT) with metabolic parameters in subjects with and without diabetes: a cross-sectional study. *Clin Endocrinol* 2008; 69(6):885-893.

(447) Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 2007; 6(5):363-375.

(448) Ridker PM. High-sensitivity C-reactive protein: Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 2001; 103(13):1813-1818.

(449) Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: From concept to clinical practice to clinical benefit. *Am Heart J* 2004; 148(1 SUPPL.):S19-S26.

(450) Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; 336(14):973-979.

(451) Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the reynolds risk score for men. *Circulation* 2008; 118(22):2243-2251.

(452) Rizkalla SW, Taghrid L, Laromiguiere M, Huet D, Huet D, Boillot J et al. Improved plasma glucose control, whole-body glucose utilization, and lipid profile on a low-glycemic index diet in type 2 diabetic men: A randomized controlled trial. *Diabetes Care* 2004; 27(8):1866-1872.

(453) Robins SJ, Collins D, Wittes JT, Papademetriou V, Deedwania PC, Schaefer EJ et al. Relation of gemfibrozil treatment and lipid levels with major coronary events. VA-HIT: A randomized controlled trial. *JAMA* 2001; 285(12):1585-1591.

(454) Roche Media Group. Roche provides update on Phase III study of dalcetrapib. 2012. 15-10-2012.
Ref Type: Online Source

(455) Rodgers A, Asia Pacific cohort Studies Collaboration. Blood Glucose and Risk of Cardiovascular Disease in the Asia Pacific Region. *Diabetes Care* 2004; 27(12):2836-2842.

(456) Rolland C, Hession M, Broom I. Effect of weight loss on adipokine levels in obese patients. *Diabetes Metab Syndr Obes* 2011; 4:315-323.

(457) Rollo J. An Account of Two Cases of the Diabetes Mellitus. 1797.

(458) Rosenson RS, Rahimtoola SH. New approaches in the intensive management of cardiovascular risk in the metabolic syndrome. *Curr Probl Cardiol* 2005; 30(5):239+241-239+279.

(459) Rossetti L, Rothman DL, DeFronzo RA, Shulman GI. Effect of dietary protein on in vivo insulin action and liver glycogen repletion. *Am J Physiol* 1989; 257(2 Pt 1):E212-E219.

(460) Rowlett R. SI Units for Clinical Data. 2001. 1-1-2006.
Ref Type: Online Source

(461) Rubio MA, Gargallo M, Millán AI, Moreno B. Drugs in the treatment of obesity: Sibutramine, orlistat and rimonabant. *Public Health Nutr* 2007; 10(10 A):1200-1205.

(462) Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD et al. Comparison of Weight-Loss Diets with Different Compositions of Fat, Protein, and Carbohydrates. *New England Journal of Medicine* 2009; 360(9):859-873.

(463) Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ et al. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 1997; 20(4):545-550.

(464) Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* 1997; 277(6):472-477.

(465) Sam S, Haffner S, Davidson MH, D'Agostino RB, Sr., Feinstein S, Kondos G et al. Relation of abdominal fat depots to systemic markers of inflammation in type 2 diabetes. *Diabetes Care* 2009; 32(5):932-937.

(466) Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J et al. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003; 348(21):2074-2081.

(467) Samaha FF, Seshadri P, Iqbal N, Stern L. Effects of a carbohydrate-restricted diet versus a fat-and calorie-restricted diet on lipid subtractions. *J Am Coll Cardiol* 2003; 41(6):243A.

(468) Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Molecular and Cellular Biology* 1994; 14(2):1431-1437.

(469) Sargrad KR, Homko C, Mozzoli M, Boden G. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin A1c, and blood pressure in patients with type 2 diabetes mellitus. *Journal of the American Dietetic Association* 2005; 105(4):573-580.

(470) Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di AE et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; 375(9733):2215-2222.

(471) Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM et al. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation* 2003; 108(4):414-419.

(472) Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-γ action in humans. *Diabetes* 2001; 50(10):2199-2202.

(473) Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* 2006; 368(9548):1660-1672.

(474) Schillaci G, Pirro M, Vaudo G, Gemelli F, Marchesi S, Porcellati C et al. Prognostic value of the metabolic syndrome in essential hypertension. *J Am Coll Cardiol* 2004; 43(10):1817-1822.

(475) Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Human nutrition Clinical nutrition* 1984; 39:5-41.

(476) Schwarzfuchs D, Golan R, Shai I. Four-Year Follow-up after Two-Year Dietary Interventions. *New England Journal of Medicine* 2012; 367(14):1373-1374.

(477) Schwenk WF, Haymond MW. Decreased uptake of glucose by human forearm during infusion of leucine, isoleucine, or threonine. *Diabetes* 1987; 36(2):199-204.

(478) Scott R, O'Brien R, Fulcher G, Pardy C, D'Emden M, Tse D et al. Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetes Care* 2009; 32(3):493-498.

(479) Scottish Intercollegiate Guidelines Network. Obesity in Scotland: Integrating Prevention with Weight Management. National Clinical Guideline 8, 1-75. 1996.
Ref Type: Pamphlet

(480) Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. *Diabetes* 2004; 53 Suppl 1:S152-S158.

(481) Sever PS, Poulter NR, Dahlöf B, Wedel H, Collins R, Beevers G et al. Reduction in cardiovascular events with atorvastatin in 2,532 patients with type 2 diabetes: Anglo-Scandinavian Cardiac Outcomes Trial-Lipid-Lowering Arm (ASCOT-LLA). *Diabetes Care* 2005; 28(5):1151-1157.

(482) Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I et al. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med* 2008; 359(3):229-241.

(483) Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I et al. Weight Loss with a Low-Carbohydrate, Mediterranean, or Low-Fat Diet. *N Engl J Med* 2008; 359(3):229-241.

(484) Shand BI, Scott RS, Elder PA, George PM. Plasma adiponectin in overweight, nondiabetic individuals with or without insulin resistance. *Diabetes Obes Metab* 2003; 5(5):349-353.

(485) Sharman MJ, Gomez AL, Kraemer WJ, Volek JS. Very low-carbohydrate and low-fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *J Nutr* 2004; 134(4):880-885.

(486) Sharman MJ, Kraemer WJ, Love DM, Avery NG, Gomez AL, Scheett TP et al. A ketogenic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. *J Nutr* 2002; 132(7):1879-1885.

(487) Sharman MJ, Volek JS. Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men. *Clin Sci (Colch)* 2004; 107(4):365-369.

(488) Shen DC, Shieh SM, Fuh MM, Wu DA, Chen YD, Reaven GM. Resistance to insulin-stimulated-glucose uptake in patients with hypertension. *J Clin Endocrinol Metab* 1988; 66(3):580-583.

(489) Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW et al. Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia. *New England Journal of Medicine* 1995; 333(20):1301-1308.

(490) Shimomura I, Funahashi T, Matsuzawa Y. Adipocytokines; cause for metabolic syndrome. *Curr Med Chem Cent Nerv Syst Agents* 2003; 3(2):121-125.

(491) Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T et al. Enhanced expression of PAI-1 in visceral fat: Possible contributor to vascular disease in obesity. *Nat Med* 1996; 2(7):800-803.

(492) Sichieri R, Coitinho DC, Leao MM, Recine E, Everhart JE. High temporal, geographic, and income variation in body mass index among adults in Brazil. *Am J Public Health* 1994; 84(5):793-798.

(493) Sidorenkov O, Nilssen O, Brenn T, Martiushov S, Arkhipovsky V, Grjibovski A. Prevalence of the metabolic syndrome and its components in Northwest Russia: the Arkhangelsk study. *BMC Public Health* 2010; 10(1):23.

(494) Sieri S, Krogh V, Berrino F, Evangelista A, Agnoli C, Brighenti F et al. Dietary glycemic load and index and risk of coronary heart disease in a large italian cohort: the EPICOR study. *Arch Intern Med* 2010; 170(7):640-647.

(495) Sieri S, Pala V, Brighenti F, Pellegrini N, Muti P, Micheli A et al. Dietary glycemic index, glycemic load, and the risk of breast cancer in an Italian prospective cohort study. *Am J Clin Nutr* 2007; 86(4):1160-1166.

(496) Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BLG, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: Correlations with insulin resistence. *Eur J Endocrinol* 2003; 149(4):331-335.

(497) Silva FM, de Almeida JC, Feoli AM. Effect of diet on adiponectin levels in blood. *Nutrition Reviews* 2011; 69(10):599-612.

(498) Sjostrom L, Rissanen A, Andersen T, Boldrin M, Golay A, Koppeschaar HP et al. Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *The Lancet* 1998; 352(9123):167-172.

(499) Skarfors ET, Lithell HO, Selinus I. Risk factors for the development of hypertension: a 10-year longitudinal study in middle-aged men. *J Hypertens* 1991; 9(3):217-223.

(500) Sloth B, Krog-Mikkelsen I, Flint A, Tetens I, Bjorck I, Vinoy S et al. No difference in body weight decrease between a low-glycemic-index and a high-glycemic-index diet but reduced LDL cholesterol after 10-wk ad libitum intake of the low-glycemic-index diet. *The American Journal of Clinical Nutrition* 2004; 80(2):337-347.

(501) Sluijs I, Beulens JWJ, van dAD, Spijkerman AMW, Grobbee DE, van der Schouw YT. Dietary Intake of Total, Animal, and Vegetable Protein and Risk of Type 2 Diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL Study. *Diabetes Care* 2010; 33(1):43-48.

(502) Snehalatha C, Yamuna A, Ramachandran A. Plasma Adiponectin Does Not Correlate With Insulin Resistance and Cardiometabolic Variables in Nondiabetic Asian Indian Teenagers. *Diabetes Care* 2008; 31(12):2374-2379.

(503) Snel M, Jonker JT, Schoones J, Lamb H, de RA, Pijl H et al. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol* 2012; 2012:983814.

(504) Sobal J, Stunkard AJ. Socioeconomic status and obesity: A review of the literature. *Psychological Bulletin* 1989; 105(2):260-275.

(505) Soderberg S, Ahren B, Jansson JH, Johnson O, Hallmans G, Asplund K et al. Leptin is associated with increased risk of myocardial infarction. *J Intern Med* 1999; 246(4):409-418.

(506) Soderberg S, Ahren B, Stegmayr B, Johnson O, Wiklund PG, Weinehall L et al. Leptin is a risk marker for first-ever hemorrhagic stroke in a population-based cohort. *Stroke* 1999; 30(2):328-337.

(507) Soderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, Alberti KG et al. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. *Int J Obes (Lond)* 2007; 31(7):1126-1133.

(508) Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. *Obes Res* 2003; 11(3):368-372.

(509) Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. *N Engl J Med* 2000; 343(1):16-22.

(510) Stears AJ, Byrne CD. Adipocyte metabolism and the metabolic syndrome. *Diabetes Obes Metab* 2001; 3(3):129-142.

(511) Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Schleicher E et al. High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. *Diabetes Care* 2007; 30(5):1173-1178.

(512) Stefansson V. Eskimos Prove An All Meat Diet Provides Excellent Health, News You Can Use, Adventures in Diet part 1. Harper's Monthly Magazine . 1-11-1935. Harper's Monthly Magazine.
Ref Type: Magazine Article

(513) Stenlöf Kaj. Recent Advances in the Use of Orlistat in the Treatment of Abdominal Obesity and Associated Cardiometabolic Risk Factors. *The Journal of Clinical Metabolism & Diabetes* 2010; 1(1):1-7.

(514) Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM et al. The hormone resistin links obesity to diabetes. *Nature* 2001; 409(6818):307-312.

(515) Steppan CM, Lazar MA. The current biology of resistin. *J Intern Med* 2004; 255(4):439-447.

(516) Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: One-year follow-up of a randomized trial. *Ann Intern Med* 2004; 140(10):778-785.

(517) Stevens VJ, Obarzanek E, Cook NR, Lee IM, Appel LJ, Smith West D et al. Long-Term Weight Loss and Changes in Blood Pressure: Results of the Trials of Hypertension Prevention, Phase II. *Annals of Internal Medicine* 2001; 134(1):1-11.

(518) Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML et al. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 2002; 45(3):369-377.

(519) Sweeney JS. Dietary factors that influence the Dextrose toleance test: A Preliminary Study. *Arch Intern Med* 1927; 40(6):818-830.

(520) Szmitko PE, Teoh H, Stewart DJ, Verma S. Adiponectin and cardiovascular disease: state of the art? *Am J Physiol Heart Circ Physiol* 2007; 292(4):H1655-H1663.

(521) Takashima N, Tomoike H, Iwai N. Retinol-binding protein 4 and insulin resistance. *N Engl J Med* 2006; 355(13):1392.

(522) Tamori Y, Sakaue H, Kasuga M. RBP4, an unexpected adipokine. *Nat Med* 2006; 12(1):30-31.

(523) Teesalu T, Kulla A, Simisker A, Siren V, Lawrence DA, Asser T et al. Tissue plasminogen activator and neuroserpin are widely expressed in the human central nervous system. *Thromb Haemost* 2004; 92(2):358-368.

(524) Teoh H, Quan A, Lovren F, Wang G, Tirgari S, Szmitko PE et al. Impaired endothelial function in C-reactive protein overexpressing mice. *Atherosclerosis* 2008; 201(2):318-325.

(525) The ACCORD study group. Effects of Combination Lipid Therapy in Type 2 Diabetes Mellitus. *New England Journal of Medicine* 2010; 362(17):1563-1574.

(526) The Scientific Advisory Committee on Nutrition. SACN Dietaary Reference Values for Energy. TSO (The Stationery Office); 2011.

(527) Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. *Cochrane Database Syst Rev* 2009;(1):CD006296.

(528) Thomas DE, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. *Cochrane Database Syst Rev* 2007;(3):CD005105.

(529) Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH et al. Exercise and Physical Activity in the Prevention and Treatment of Atherosclerotic Cardiovascular Disease: A Statement From the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 2003; 107(24):3109-3116.

(530) Thompson WG, Holdman NR, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of Energy-Reduced Diets High in Dairy Products and Fiber on Weight Loss in Obese Adults. *Obesity Research* 2005; 13(8):1344-1353.

(531) Thomssen C, Oppelt P, Janicke F, Ulm K, Harbeck N, Hofler H et al. Identification of low-risk node-negative breast cancer patients by tumor biological factors PAI-1 and cathepsin L. *Anticancer Res* 1998; 18(3C):2173-2180.

(532) Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WHM et al. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome[mdash]LIPGENE: a European randomized dietary intervention study. *Int J Obes* 2011; 35(6):800-809.

(533) Tina V, Mikkel C, Anders E Junker, Filip KK, Lise LG. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2012; 344.

(534) Toplak H, Ziegler O, Keller U, Hamann A, Godin C, Wittert G et al. X-PERT: Weight reduction with orlistat in obese subjects receiving a mildly or moderately reduced-energy diet. Early response to treatment predicts weight maintenance. *Diabetes Obes Metab* 2005; 7(6):699-708.

(535) Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L. XENical in the Prevention of Diabetes in Obese Subjects (XENDOS) Study. *Diabetes Care* 2004; 27(1):155-161.

(536) Toruner F, Altinova AE, Bukan N, Arslan E, Akbay E, Ersoy R et al. Plasma visfatin concentrations in subjects with type 1 diabetes mellitus. *Horm Res* 2009; 72(1):33-37.

(537) Trayhurn P, Wood IS. Signalling role of adipose tissue: Adipokines and inflammation in obesity. *Biochem Soc Trans* 2005; 33(5):1078-1081.

(538) Tremblay A, Després J-P, Maheux J, Pouliot MC, Nadeau A, Moorjani S et al. Normalization of the metabolic profile in obese women by exercise and a low fat diet. *Med Sci Sports Exerc* 1991; 23(12):1326-1331.

(539) Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. *Annu Rev Nutr* 2007; 27:293-310.

(540) Tribble DL, Chu BM, Gong EL, van Venrooij F, Nichols AV. HDL antioxidant effects as assessed using a nonexchangeable probe to monitor particle-specific peroxidative stress in LDL-HDL mixtures. *J Lipid Res* 1995; 36(12):2580-2589.

(541) Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A et al. Elevation of Free Fatty Acids Induces Inflammation and Impairs Vascular Reactivity in Healthy Subjects. *Diabetes* 2003; 52(12):2882-2887.

(542) Tschoner A, Sturm W, Engl J, Kaser S, Laimer M, Laimer E et al. Retinol-binding Protein 4, Visceral Fat, and the Metabolic Syndrome: Effects of Weight Loss. *Obesity* 2008; 16(11):2439-2444.

(543) Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; 344(18):1343-1350.

(544) Turner R, Murchison L, Wright AD, Oakley N, Kohner E, Hayes R et al. United Kingdom prospective diabetes study 24: A 6-year, randomized, controlled trial comparing sulfonylurea, insulin, and metformin therapy in patients with newly diagnosed type 2 diabetes that could not be controlled with diet therapy. *Ann Intern Med* 1998; 128(3):165-175.

(545) Unger J. Incretins: clinical perspectives, relevance, and applications for the primary care physician in the treatment of patients with type 2 diabetes mellitus. *Mayo Clin Proc* 2010; 85(12 Suppl):S38-S49.

(546) Unger RH, Eisentraut AM. Entero-insular axis. *Arch Intern Med* 1969; 123(3):261-266.

(547) Unick JL, Beavers D, Jakicic JM, Kitabchi AE, Knowler WC, Wadden TA et al. Effectiveness of lifestyle interventions for individuals with severe obesity and type 2 diabetes: results from the Look AHEAD trial. *Diabetes Care* 2011; 34(10):2152-2157.

(548) Valsamakis G, McTernan PG, Chetty R, Al Daghri N, Field A, Hanif W et al. Modest weight loss and reduction in waist circumference after medical treatment are associated with favorable changes in serum adipocytokines. *Metabolism* 2004; 53(4):430-434.

(549) Van Dam RM, Visscher AW, Feskens EJ, Verhoef P, Kromhout D. Dietary glycemic index in relation to metabolic risk factors and incidence of coronary heart disease: the Zutphen Elderly Study. *Eur J Clin Nutr* 2000; 54(9):726-731.

(550) Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 2005; 365(9468):1389-1397.

(551) Van Gaal LF, Scheen AJ, Rissanen AM, Rossner S, Hanotin C, Ziegler O. Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. *Eur Heart J* 2008; 29(14):1761-1771.

(552) Van Hoek M, Van Tol A, Van Vark-van der Zee, Jansen H, Kastelein JJP, Sijbrands EJG et al. Role of plasma adiponectin on the HDL-cholesterol raising effect of atorvastatin in patients with type 2 diabetes*. *Curr Med Res Opin* 2008; 25(1):93-101.

(553) Varady KA, Tussing L, Bhutani S, Braunschweig CL. Degree of weight loss required to improve adipokine concentrations and decrease fat cell size in severely obese women. *Metabolism* 2009; 58(8):1096-1101.

(554) Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee M-J et al. Human visfatin expression: Relationship to insulin sensitivity, intramyocellular lipids, and inflammation. *J Clin Endocrinol Metab* 2007; 92(2):666-672.

(555) Vendrell J, Broch M, Vilarrasa N, Molina A, Gomez JM, Gutierrez C et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obes Res* 2004; 12(6):962-971.

(556) Venner T. Via Recta Ad Vitam Longam: Or, A Plaine Philosophicall Demonstration of the Nature, Faculties, and Effects of All Such Things as by Way of Nourishments Make for the Preservation of Health, with Divers Necessary Dieticall Observations; as Also of the True Use and Effects of Sleepe, Exercise, Excretions and Preturbations, with Just Applications to Every Age, Constitution of Body, and Time of Yeere. Imprinted by Felix Kyngston, for Richard Moore, and are to be sold at his shop in Saint Dunstans Church-yard in Fleetstreet; 1628.

(557) Venugopal SK, Devaraj S, Jialal I. C-reactive protein decreases prostacyclin release from human aortic endothelial cells. *Circulation* 2003; 108(14):1676-1678.

(558) Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 2001; 44(3):312-319.

(559) Vettor R, Serra R, Fabris R, Pagano C, Federspil G. Effect of Sibutramine on Weight Management and Metabolic Control in Type 2 Diabetes. *Diabetes Care* 2005; 28(4):942-949.

(560) Vgontzas AN, Legro RS, Bixler EO, Grayev A, Kales A, Chrousos GP. Polycystic ovary syndrome is associated with obstructive sleep apnea and daytime sleepiness: Role of insulin resistance. *J Clin Endocrinol Metab* 2001; 86(2):517-520.

(561) Volek JS, Sharman MJ, Gomez AL, DiPasquale C, Roti M, Pumerantz A et al. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *J Am Coll Nutr* 2004; 23(2):177-184.

(562) Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation* 2001; 104(25):3052-3056.

(563) Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *The Lancet* 2001; 358(9298):2026-2033.

(564) Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Kelly A, Rumley A et al. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* 2007; 191(2):418-426.

(565) Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr* 2005; 82(1):41-48.

(566) Weikert C, Westphal S, Berger K, Dierkes J, Möhlig M, Spranger J et al. Plasma resistin levels and risk of myocardial infarction and ischemic stroke. *J Clin Endocrinol Metab* 2008; 93(7):2647-2653.

(567) Weiss R, Caprio S. The metabolic consequences of childhood obesity. *Best Pract Res Clin Endocrinol Metab* 2005; 19(3):405-419.

(568) Westman EC, Mavropoulos J, Yancy WS, Jr., Volek JS. A review of low-carbohydrate ketogenic diets. *Curr Atheroscler Rep* 2003; 5(6):476-483.

(569) Westman EC, Yancy Jr WS, Mavropoulos JC, Marquart M, McDuffie JR. The effect of a low-carbohydrate, ketogenic diet versus a low-glycemic index diet on glycemic control in type 2 diabetes mellitus. *Nutr Metab (Lond)* 2008; 5:36.

(570) Wilcox I, McNamara SG, Collins FL, Grunstein RR, Sullivan CE. 'Syndrome Z': The interaction of sleep apnoea, vascular risk factors and heart disease. *Thorax* 1998; 53(Suppl 3):S25-S28.

(571) Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. *Am J Clin Nutr* 2002; 76(1):274S-280S.

(572) Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF et al. British hypertension society guidelines for hypertension management 2004 (BHS-IV): Summary. *Br Med J* 2004; 328(7440):634-640.

(573) Wing RR, Jeffery RW. Effect of modest weight loss on changes in cardiovascular risk factors: Are there differences between men and women or between weight loss and maintenance? *Int J Obesity* 1995; 19(1):67-73.

(574) Wing RR, Venditti E, Jakicic JM, Polley BA, Lang W. Lifestyle intervention in overweight individuals with a family history of diabetes. *Diabetes Care* 1998; 21(3):350-359.

(575) Wolever TM, Bolognesi C. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *J Nutr* 1996; 126(11):2807-2812.

(576) Wolever TM, Gibbs AL, Mehling C, Chiasson JL, Connelly PW, Josse RG et al. The Canadian Trial of Carbohydrates in Diabetes (CCD), a 1-y controlled trial of low-glycemic-index dietary carbohydrate in type 2 diabetes: no effect on glycated hemoglobin but reduction in C-reactive protein. *The American Journal of Clinical Nutrition* 2008; 87(1):114-125.

(577) Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense HW, Joffres M et al. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *JAMA* 2003; 289(18):2363-2369.

(578) Wolfe BE, Jimerson DC, Orlova C, Mantzoros CS. Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. *Clin Endocrinol* 2004; 61(3):332-338.

(579) Wood D, Wray R, Poulter N, Williams B, Kirby M, Patel V et al. JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 2005; 91(SUPPL. 5):v1-v52.

(580) Wood PD, Stefanick ML, Williams PT, Haskell WL. The effects on plasma lipoproteins of a prudent weight-reducing diet, with or without exercise, in overweight men and women. *N Engl J Med* 1991; 325(7):461-466.

(581) Wood PD, Terry RB, Haskell WL. Metabolism of substrates: diet, lipoprotein metabolism, and exercise. *Fed Proc* 1985; 44(2):358-363.

(582) World Health Organisation. Obesity and Overweight. Fact sheet N°311 [WHO Media centre]. 2011.
Ref Type: Pamphlet

(583) World Health Organisation. Global Database on Body Mass Index. 2012. 8-4-2012.
Ref Type: Online Source

(584) Wright JD, National Center for Health Statistics (, Centers for Disease Control and Prevention (. Dietary Intake of Ten Key Nutrients for Public Health, United States, 1999-2000. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2003.

(585) Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; 7(8):941-946.

(586) Yan LL, Liu K, Matthews KA, Daviglus ML, Ferguson TF, Kiefe CI. Psychosocial factors and risk of hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *JAMA* 2003; 290(16):2138-2148.

(587) Yancy WS, Jr., Foy M, Chalecki AM, Vernon MC, Westman EC. A low-carbohydrate, ketogenic diet to treat type 2 diabetes. *Nutr Metab* 2005; 2(34).

(588) Yancy WS, Jr., Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: A randomized, controlled trial. *Ann Intern Med* 2004; 140(10):769-777.

(589) Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005; 436(7049):356-362.

(590) Yang R-Z, Huang Q, Xu A, McLenithan JC, Eisen JA, Shuldiner AR et al. Erratum: Comparative studies of resistin expression and phylogenomics in human and mouse (Biochemical and Biophysical Research Communications (2003) 310 (927-935) PII: S0006291X03018928). *Biochem Biophys Res Commun* 2003; 312(3):866.

(591) Yang W-S, Lee W-J, Funahashi T, Tanaka S, Matsuzawa Y, Chao C-L et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 2001; 86(8):3815-3819.

(592) Yannakoulia M, Yiannakouris N, Blüher S, Matalas A-L, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003; 88(4):1730-1736.

(593) Yesilbursa D, Serdar A, Heper Y, Sarac M, Coskun S, Kazazoglu AR et al. The effect of orlistat-induced weight loss on interleukin-6 and C-reactive protein levels in obese subjects. *Acta Cardiol* 2005; 60(3):265-269.

(594) Yki-Jarvinen H, Nikkila K, Makimattila S. Metformin prevents weight gain by reducing dietary intake during insulin therapy in patients with type 2 diabetes mellitus. *Drugs* 1999; 58(SUPPL. 1):53-54.

(595) You T, Yang R, Lyles MF, Gong D, Nicklas BJ. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. *Am J Physiol Endocrinol Metab* 2005; 288(4):E741-E747.

(596) Yudkin JS, Juhan-Vague I, Hawe E, Humphries SE, Di Minno G, Margaglione M et al. Low-grade inflammation may play a role in the etiology of the metabolic syndrome in patients with coronary heart disease: The HIFMECH study. *Metabolism* 2004; 53(7):852-857.

(597) Yusuf PS, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet* 2004; 364(9438):937-952.

(598) Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; 342(3):145-153.

(599) Zhang Y-Y, Gottardo L, Thompson R, Powers C, Nolan D, Duffy J et al. A visfatin promoter polymorphism is associated with low-grade inflammation and type 2 diabetes. *Obesity* 2006; 14(12):2119-2126.

(600) Zheng D, Wooter MH, Zhou Q, Dohm GL. The effect of exercise on ob gene expression. *Biochem Biophys Res Commun* 1996; 225(3):747-750.

(601) Zhong M, Tan H-W, Gong H-P, Wang S-F, Zhang Y, Zhang W. Increased serum visfatin in patients with metabolic syndrome and carotid atherosclerosis. *Clin Endocrinol* 2008; 69(6):878-884.

(602) Zhu S, St Onge M-P, Heshka S, Heymsfield SB. Lifestyle behaviors associated with lower risk of having the metabolic syndrome. *Metabolism* 2004; 53(11):1503-1511.

(603) Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002; 105(7):804-809.

(604) Zou C, Shao J. Role of adipocytokines in obesity-associated insulin resistance. *J Nutr Biochem* 2008; 19(5):277-286.